USER GUIDE

applied biosystems® by life technologies™

GlobalFiler™ Express PCR Amplification Kit

for use with:
200 reaction kit (Part no. 4476609)
1000 reaction kit (Part no. 4474665)

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Revision Date October 2012

life technologies™

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TRADEMARKS

Contents

About This Guide

IMPORTANT! Before using this product, read and understand the information the "Safety" appendix in this document.

Revision history

Revision	Date	Description
A	October 2012	New document.

Purpose

The *GlobalFiler™ Express PCR Amplification Kit User Guide* provides information about the Life Technologies instruments, chemistries, and software associated with the GlobalFiler™ Express PCR Amplification Kit.

1 Overview

Product overview

Purpose

The GlobalFiler™ Express PCR Amplification Kit is a 6-dye, short tandem repeat (STR) multiplex assay optimized to allow direct amplification from the following types of single-source samples:

- Blood and buccal samples on treated paper substrates without the need for sample purification.
- Blood and buccal samples collected on untreated paper substrates and treated with Prep-n-Go™ Buffer.
- Buccal samples collected on swab substrates and treated with Prep-n-Go™ Buffer

The GlobalFiler™ Express Kit amplifies 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, and the sex-determining marker, Amelogenin, 1 Y STR locus, DYS391, and 1 Y insertion/deletion (Y indel) locus in a single PCR reaction (24 loci total).

Substrate examples

- Treated paper: Copan NUCLEIC-CARD™ system or Whatman FTA® cards
- Untreated paper: Bode Buccal DNA Collector™ or 903 paper
- Swab: Copan FLOQSwabs™

Product description

The GlobalFiler™ Express Kit contains all the necessary reagents for the amplification of human genomic DNA.

The reagents are designed for use with the following instruments and software:

- Veriti® 96-Well Thermal Cycler (Part no. 4375786)

  IMPORTANT! The GlobalFiler™ Express Kit is validated for use with the Veriti® 96-well Thermal Cycler (Part no. 4375786) NOT the Veriti® 96-Well Fast Thermal Cycler (Part no. 4375305). Please ensure you are using the correct Veriti® Thermal Cycler model.

- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block

- 3500/3500xL Genetic Analyzer (requires 3500 Data Collection Software v1 or v2 and HID Updater 3500 Data Collection v2)
- 3130/3130*xl* Genetic Analyzer (requires Data Collection Software v4 and 3130/3730 Data Collection v4 6-Dye Module v1)
- 3730 Genetic Analyzer (requires Data Collection Software v4 and 3130/3730 Data Collection v4 6-Dye Module v1)
- GeneMapper® *ID-X* Software v1.4

About the primers

The GlobalFiler™ Express Kit uses the same primer sequences as the NGM™ SElect Express and the Identifiler® Direct Kits, which include SNP-specific primers for the vWA, D16S539, Amel, D2S441, D22S1045, and D8S1179 loci. The GlobalFiler™ Express Kit uses the same primer synthesis and purification improvements as the NGM™ SElect Express and the Identifiler® Direct Kits, which enhance the assay signal-to-noise ratio and simplify the interpretation of results.

The GlobalFiler™ Express Kit also includes the following primer additions and modifications:

- Addition of DYS391 and a novel Y indel.
- The TPOX reverse primer has been redesigned to relocate the amplicon into the higher size range of the multiplex and optimize marker spacing.
- Addition of 8 new SNP-specific primers for the D3S1358, vWA, D18S51, D19S433, TH01, FGA, D5S818, and SE33 loci. The second degenerate primer was added to the vWA locus to address two different SNPs found in the primer binding site.

Non-nucleotide linkers are used in primer synthesis for the following loci: D19S433, vWA, CSF1PO, D2S441, TH01, FGA, and D12S391. For these primers, non-nucleotide linkers are placed between the primers and the fluorescent dye during oligonucleotide synthesis (Butler 2005, Grossman *et al.*, 1994). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate inter-locus spacing. The combination of a six-dye fluorescent system and the inclusion of non-nucleotide linkers allows for simultaneous amplification and efficient separation of all 24 markers during automated DNA fragment analysis.

Loci amplified by the kit

Table 1 shows the loci amplified, their chromosomal locations, and the corresponding fluorescent marker dyes. The GlobalFiler™ Express Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder, and the genotype of the DNA Control 007, are also listed in the table.

Table 1 GlobalFiler™ Express PCR Amplification Kit loci and alleles

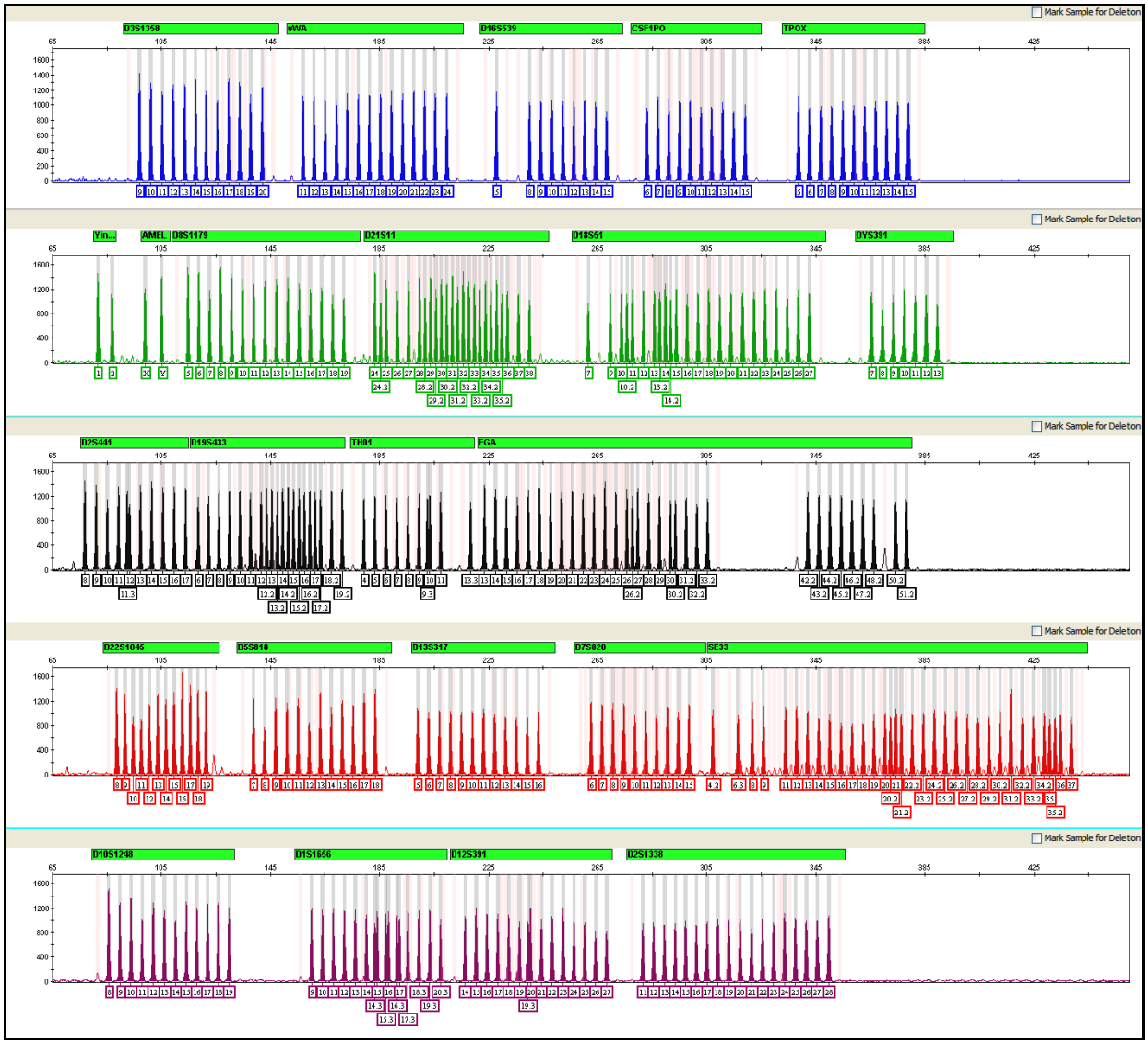
Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
D3S1358	3p21.31	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	6-FAM™	15, 16
vWA	12p13.31	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		14, 16
D16S539	16q24.1	5, 8, 9, 10, 11, 12,13, 14, 15		9, 10
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		11, 12
TPOX	2p23-2per	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15		8, 8
Y indel	Y	1, 2	VIC®	2
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y		X, Y
D8S1179	8q24.13	5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19		12, 13
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38		28, 31
D18S51	18q21.33	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		12, 15
DYS391	Y	7, 8, 9, 10, 11, 12, 13		11
D2S441	2p14	8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17	NED™	14, 15
D19S433	19q12	6, 7, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		7, 9.3
FGA	4q28	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		24, 26
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	TAZ™	11, 16
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18		11, 11
D13S317	13q22-31	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11, 11
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		7, 12
SE33	6q14	4.2, 6.3, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37		17, 25.2

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
D10S1248	10q26.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	SID™	12, 15
D1S1656	1q42.2	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3		13, 16
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27		18, 19
D2S1338	2q35	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		20, 23

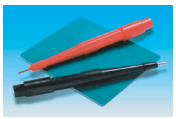
Allelic ladder

Figure 1 shows the allelic ladder for the GlobalFiler™ Express Kit. See "Allelic ladder requirements" on page 30 for information on ensuring accurate genotyping.

Figure 1 GeneMapper® *ID-X* Software plot of the GlobalFiler™ Express Allelic Ladder



Workflow

<table>
<tr><td rowspan="4">Perform PCR</td><td>Obtain samples</td><td>Treated or untreated paper substrates</td><td>Obtain samples</td><td>Swab substrates</td></tr>
<tr><td>Prepare samples</td><td> 
Harris Manual Punch

 
BSD600 Semi-Automated Dried Sample Punch Instrument</td><td>Prepare samples</td><td> 
Lyse in Prep-n-Go™ Buffer</td></tr>
<tr><td>Prepare reactions</td><td> 
Untreated paper only: Prep-n-Go™ Buffer

GlobalFiler™ Express PCR Amplification Kit</td><td>Prepare reactions</td><td>GlobalFiler™ Express PCR Amplification Kit</td></tr>
<tr><td>Perform PCR</td><td> 
Veriti® 96-Well Thermal Cycler</td><td colspan="2"> 
GeneAmp® PCR System 9700 Cycler</td></tr>
<tr><td>Perform electro-phoresis</td><td colspan="3"> 
3500/3500xL Genetic Analyzer

 
3130/3130xl Genetic Analyzer

 
3730 Genetic Analyzer</td></tr>
<tr><td>Analyze data</td><td colspan="3"> 
GeneMapper® ID-X Software</td></tr>
</table>

Instrument and software overview

This section provides information about the data collection and analysis software versions required to run the this kit on specific instruments.

Data collection and analysis software

The data collection software provides instructions to firmware running on the instrument and displays instrument status and raw data in real time. As the instrument measures sample fluorescence with its detection system, the data collection software collects the data and stores it. The data collection software stores information about each sample in a sample file (.fsa files for 31xx or 3730 instruments and .hid files for 3500 instruments), which is then analyzed by the analysis software.

Instrument and software compatibility

Instrument	Operating system	Data collection software	Additional software	Analysis software
3500†/ 3500xL†	Windows Vista®	3500 Series Data Collection Software v1	HID Updater 3500 DC v2.0 (Part no. 4480670)	GeneMapper® *ID-X* Software v1.4 • Windows® XP *or* • Windows® 7
	Windows® 7	3500 Series Data Collection Software v2		
3130/ 3130*xl*†	Windows® 7	Data Collection Software v4	DC v4 6-Dye Module v1 License (Contact Life Technologies)	
3730				

† We conducted validation studies for the GlobalFiler™ Express Kit using the 3130*xl*, 3500, and 3500xL configurations.

About multicomponent analysis

Applied Biosystems® fluorescent multi-color dye technology allows the analysis of multiple loci, including loci that have alleles with overlapping size ranges. Alleles for overlapping loci are distinguished by labeling locus-specific primers with different colored dyes.

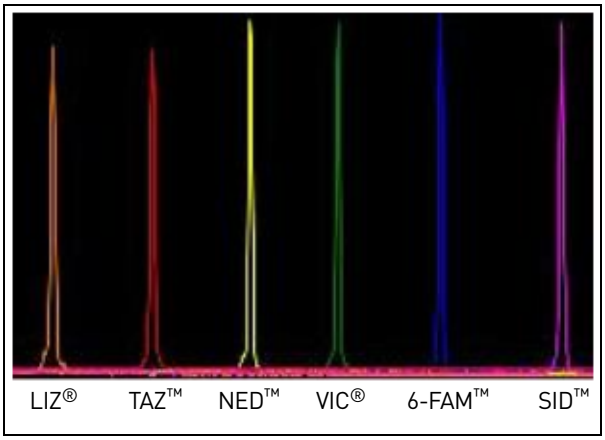
Multicomponent analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The five dyes used in the GlobalFiler™ Express Kit to label samples are 6-FAM™, VIC®, NED™, TAZ™, and SID™ dyes. The sixth dye, LIZ®, is used to label the GeneScan™ 600 LIZ® Size Standard v2.0.

How multicomponent analysis works

Each of these fluorescent dyes emits its maximum fluorescence at a different wavelength. During data collection on the Applied Biosystems® instruments, the fluorescence signals are separated by a diffraction grating according to their wavelengths and projected onto a charge-coupled device (CCD) camera in a predictably spaced pattern. The 6-FAM™ dye emits at the shortest wavelength and is displayed as blue, followed by the VIC® dye (green), NED™ dye (yellow), TAZ™ dye (red), SID™ dye (purple), and LIZ® dye (orange).

Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes (Figure 2). The goal of multicomponent analysis is to correct for spectral overlap.

Figure 2 Spectral calibration of the six dyes used in the GlobalFiler™ Express Kit



Materials and equipment

Kit contents and storage

The GlobalFiler™ Express Kit contains sufficient quantities of the following reagents to perform 200 (Part no. 4476609) or 1000 (Part no. 4474665) amplifications at 15 µL/amplification:

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze/thaw cycles to a minimum.

Table 2 Kit Contents and Storage

Component	Description	200 reaction	1000 reaction	Storage
GlobalFiler™ Express Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, enzyme, and 0.05% sodium azide in buffer and salt.	1 tube, 1.2 mL	1 bottle, 6 mL	–15 to –25°C upon receipt, 2 to 8°C after initial use for up to 1 month
GlobalFiler™ Express Primer Set	Contains forward and reverse primers to amplify human DNA targets.	1 tube, 1.2 mL	1 bottle, 6 mL	
GlobalFiler™ Express Allelic Ladder	Contains amplified alleles. See Table 1 on page 13 for a list of alleles included in the allelic ladder.	1 tube, 0.065 mL	1 tube, 0.15 mL	
DNA Control 007	Contains 2 ng/µL human male genomic DNA in 0.05% sodium azide and buffer.† See Table 1 on page 13 for profile.	1 tube, 0.05 mL	2 tubes, 0.05 mL/tube	

† The DNA Control 007 is included at a concentration appropriate to its intended use as an amplification control (i.e., to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). The DNA Control 007 is not designed to be used as a DNA quantitation control and laboratories may expect to see variation from the labelled concentration when quantitating aliquots of the DNA Control 007.

Standards for samples

For the GlobalFiler™ Express Kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **DNA Control 007** – A positive control for evaluating the efficiency of the amplification step and STR genotyping using the GlobalFiler™ Express Allelic Ladder.
- **GeneScan™ 600 LIZ® Size Standard v2.0** – Used for obtaining sizing results. This standard, which has been evaluated as an internal size standard, yields precise sizing results for GlobalFiler™ Express Kit PCR products. Order the GeneScan™ 600 LIZ® Size Standard v2.0 (Part no. 4408399) separately.
- **GlobalFiler™ Express Allelic Ladder** – Developed for accurate characterization of the alleles amplified by the GlobalFiler™ Express Kit. The Allelic Ladder allows for automatic genotyping of the majority of reported alleles for the 24 loci. See page 13 for a list of the alleles included in the Allelic Ladder.

2 Perform PCR

Optimize PCR cycle number

Before using the GlobalFiler™ Express Kit for the first time, perform a single initial sensitivity experiment to determine the appropriate cycle number to use during internal validation studies and operational use of the GlobalFiler™ Express Kit. This experiment accounts for instrument-to-instrument and sample-to-sample variations. If you are processing multiple sample type and substrate combinations (for example, buccal samples on treated paper and buccal samples on swabs), perform separate sensitivity experiments for each sample type and substrate to be used for testing.

Select samples and prepare plates

1. Select 26 of each sample+substrate type. Ensure the selected samples represent a "typical" range of samples analyzed in your laboratory.

   IMPORTANT! The number of samples recommended for this study has been chosen to allow you to complete electrophoresis using a single 96-well plate, thus minimizing the impact of run-to-run variation on the results. Examples of PCR and electrophoresis plate layouts are provided on page 73.

2. Prepare the samples and the reactions as described in the appropriate protocols later in this chapter. Prepare sufficient PCR reagents to complete amplification of three replicate plates.
3. Create three identical PCR plates (see page 73 for a suggested plate layout).
4. Amplify each plate using a different cycle number to determine the optimum conditions for use in your laboratory. Suggested cycle numbers for different sample type and substrate combinations are listed below.

Sample type	Substrate		
	Treated paper	Untreated paper	Swab
Blood	25, 26, 27 cycles	25, 26, 27 cycles	N/A
Buccal	26, 27, 28 cycles	26, 27, 28 cycles	25, 26, 27 cycles

Note: Our testing has not included blood samples on swab substrates. This sample type is not frequently used for the collection of database or casework reference samples.

Note: To minimize the effect of instrument-to-instrument variation, use the same thermal cycler to amplify all three plates. To maximize result quality, prepare and amplify Plate 1 then repeat for Plates 2 and 3. Do not prepare all three plates simultaneously.

Determine optimum conditions

1. Run the PCR products on the appropriate CE platform using the recommended protocol; see Chapter 3, "Perform Electrophoresis" on page 29.
2. Based on the results of the sensitivity study, select the appropriate PCR cycle number for future experiments.

Our studies indicate the optimum PCR cycle number should generate profiles with the following heterozygote peak heights, with no instances of allelic dropout and minimal occurrence of off-scale allele peaks:

Instrument	Heterozygous peak height
3500 Series	3000–12,000 RFU
3130 Series	1000–3000 RFU
3730	3000–12,000 RFU

The GlobalFiler™ Express Kit is optimized to amplify unpurified:

- Single-source blood samples on treated paper or untreated paper
- Buccal samples on treated paper, untreated paper, or swabs

When amplifying single-source, unpurified samples using the GlobalFiler™ Express Kit, you should expect to see greater variation in peak height from sample to sample than is expected with purified samples. Careful optimization of the cycle number will help to minimize this variation.

Treated paper substrates: prepare reactions

Sample prep guidelines

- Do not add water to the wells on the reaction plate before adding the punches. If your laboratory is experiencing static issues with the paper discs, you may prepare and dispense the 15 µL reaction mix into the wells of the reaction plate before adding the punches.
- Make the punch as close as possible to the center of the sample to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.
- For manual punching: Place the tip of a 1.2 mm Harris Micro-Punch on the card, hold the barrel of the Harris Micro-Punch (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch in to the appropriate well on the reaction plate.
- For automated punching: Refer to the User Guide of your automated or semi-automated disc punch instrument for proper guidance.

Prepare low-TE buffer

You can prepare the buffer as described below or order it from Teknova (Cat # T0223).

To prepare low-TE buffer:

1. Mix together:
   - 10 mL of 1 M Tris-HCl, pH 8.0
   - 0.2 mL of 0.5 M EDTA, pH 8.0
   - 990 mL glass-distilled or deionized water

   Note: Adjust the volumes accordingly for specific needs.
2. Aliquot and autoclave the solutions.
3. Store at room temperature.

Prepare the reactions

1. Add samples to the reaction plate:

To these well(s) of a MicroAmp® Optical 96-Well Reaction Plate...	Add:	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control	• For 25 and 26 cycles	3 µL of DNA Control 007
	• For 27 cycles	2 µL of DNA Control 007
	• For 28 cycles	1 µL of DNA Control 007

IMPORTANT! Do not add a blank disc to the positive control well.

Note: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.

2. Calculate the volume of each component needed to prepare the reactions, using the table below.

Reaction component	Volume per reaction
Master Mix	6.0 µL
Primer Set	6.0 µL
Low TE buffer	3.0 µL

Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! This kit has been optimized for a 15-µL PCR reaction volume to overcome the PCR inhibition expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

3. Prepare reagents. Thaw the Master Mix and the Primer Set, then vortex 3 seconds and centrifuge briefly before opening the tubes or bottles.

   IMPORTANT! Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8 °C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

4. Pipet the required volumes of components into an appropriately sized polypropylene tube.
5. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
6. Dispense 15 µL of the reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate.
7. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

   IMPORTANT! We recommend adhesive film for plate sealing to provide a consistent seal across all wells and prevent evaporation. Do not use caps, which may not provide a consistent seal across all wells.

   IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block, place a MicroAmp® compression pad (Part no. 4312639) on top of the plate to additionally prevent evaporation during thermal cycling. The Veriti® Thermal Cycler does not require a compression pad.

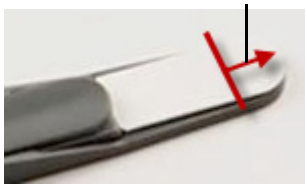
8. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.
9. Amplify the samples in a Veriti® 96-well Thermal Cycler or PCR System 9700 with the silver or gold-plated silver 96-well block as described in "Perform PCR" on page 28.

   IMPORTANT! This kit is not validated for use with the GeneAmp PCR System 9700 with the aluminium 96-well block. Use of this thermal cycling platform may adversely affect performance of this kit.

Untreated paper substrates: prepare reactions

Sample prep guidelines

- If you are processing a blood sample collected on untreated paper substrate, make the punch as close as possible to the center of the sample to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.
- If you are using a Bode Buccal DNA Collector™, make the punch as close as possible to the tip of the DNA collector to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.



- For manual punching: Place the tip of a 1.2 mm Harris Micro-Punch on the card, hold the barrel of the Harris Micro-Punch (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch in to the appropriate well on the reaction plate.
- For automated punching: Please refer to the User Guide of your automated or semi-automated disc punch instrument for proper guidance.

Prepare the reactions

1. Add Prep-n-Go™ Buffer (Part no. 4467079) to the reaction plate:

To these well(s) of a MicroAmp® Optical 96-Well Reaction Plate...	Add:	
Negative control	3 µL of Prep-n-Go™ Buffer	
Test samples	3 µL of Prep-n-Go™ Buffer	
Positive control	• For 25 and 26 cycles	0 µL of Prep-n-Go™ Buffer
	• For 27 cycles	1 µL of Prep-n-Go™ Buffer
	• For 28 cycles	2 µL of Prep-n-Go™ Buffer

2. Add samples to the reaction plate:

To these well(s) of a MicroAmp® Optical 96-Well Reaction Plate...	Add:	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control **IMPORTANT!** Do not add a blank disc to the positive control well.	• For 25 and 26 cycles	3 µL of DNA Control 007
	• For 27 cycles	2 µL of DNA Control 007
	• For 28 cycles	1 µL of DNA Control 007
Note: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.		

3. Centrifuge the plate to ensure the punches are immersed in the Prep-n-Go™ Buffer.

4. Calculate the volume of each component needed to prepare the reactions, using the table below.

Reaction component	Volume per reaction
Master Mix	6.0 µL
Primer Set	6.0 µL

Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! This kit has been optimized for a 15-µL PCR reaction volume to overcome the PCR inhibition expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

5. Prepare reagents. Thaw the Master Mix and the Primer Set, then vortex for 3 seconds and centrifuge briefly before opening the tubes or bottles.

IMPORTANT! Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8 °C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

6. Pipet the required volumes of components into an appropriately sized polypropylene tube.
7. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
8. Dispense 12 µL of the reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate. The final volume in each well is 15 µL (reaction mix plus Prep-n-Go™ Buffer and sample or positive control).
9. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

IMPORTANT! We recommend adhesive film for plate sealing to provide a consistent seal across all wells and prevent evaporation. Do not use caps, which may not provide a consistent seal across all wells.

IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block, place a MicroAmp® compression pad (Part no. 4312639) on top of the plate to additionally prevent evaporation during thermal cycling. The Veriti® Thermal Cycler does not require a compression pad.

10. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.

11. Amplify the samples in a Veriti® 96-well Thermal Cycler or PCR System 9700 with the silver or gold-plated silver 96-well block as described in "Perform PCR" on page 28.

IMPORTANT! This kit is not validated for use with the GeneAmp PCR System 9700 with the aluminium 96-well block. Use of this thermal cycling platform may adversely affect performance of the this kit.

Swab substrates: prepare reactions

Sample prep guidelines

- Detach each buccal swab head from the swab shaft before lysis.
- If using the heated lysis protocol, perform lysis in either of the following formats:
  - 1.5 mL tubes with a heat block (VWR® Scientific Select dry heat block or similar)
  - 96-well deep-well plate (Part no. 4392904) with an oven and a metal plate adaptor (Robbins Scientific® Model 400 Hybridization Incubator or similar, Agilent® Benchtop Rack for 200 µl Tubes/V Bottom Plates (metal) Part no. 410094 or similar)

    IMPORTANT! Do not use a plastic plate adaptor.
- For optimum performance, lysis of a whole swab is recommended. To preserve the sample, evaluate lysis of a half swab.

Prepare the sample lysate (room temperature protocol)

1. Add 400 µL Prep-n-Go™ Buffer (Part. no. 4471406) to 1.5 mL tubes or the appropriate wells of a 96-well deep-well plate (Part no. 4392904).
2. Into each tube or well, put the entire head of each swab and let stand for 20 minutes at room temperature (20 to 25°C) to lyse the sample.
3. After 20 minutes, transfer the sample lysate out of the sample plate into tubes or plates for storage, then discard the deep-well plate containing the swab heads.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate plate before transferring the lysate.
4. Proceed to "Prepare the reactions" on page 21 or see "Store the sample lysate" on page 27.

Prepare the sample lysate (heat protocol)

This protocol may improve the performance for challenging or aged samples.

1. Preheat the heat block to 90°C or the oven with metal plate adaptor to 99°C.
2. Add 400 µL Prep-n-Go™ Buffer (for buccal swabs, Part. no. 4471406) to 1.5 mL tubes or the appropriate wells of a 96-well deep-well plate (Part no. 43929040).
3. Into each tube or well, put the entire head of each swab. If you are using tubes, cap the tubes. Let the tubes or plate stand for 20 minutes in the preheated heat block or oven to lyse the sample.
4. After 20 minutes, remove the tubes or the deep-well plate from the heat block or oven.

5. Let the lysate stand at room temperature for at least 15 minutes to cool the lysate (for accurate pipetting).
6. Transfer the sample lysate out of the 1.5 mL tubes or sample plate into tubes or plates for storage, then discard the 1.5 mL tubes or deep-well plate containing the swab heads.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate plate before transferring the lysate.
7. Proceed to the next section to prepare the reactions or see "Store the sample lysate" on page 27.

Prepare the reactions

1. Add Prep-n-Go™ Buffer (Part no. 4471406) to the control wells in the reaction plate:

Well(s)	Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate...	
Negative control	3 µL of Prep-n-Go™ Buffer	
Positive control	• For 25 and 26 cycles	0 µL of Prep-n-Go™ Buffer
	• For 27 cycles	1 µL of Prep-n-Go™ Buffer
	• For 28 cycles	2 µL of Prep-n-Go™ Buffer

2. Calculate the volume of each component needed to prepare the reactions using the table below.

Reaction component	Volume per reaction
Master Mix	6.0 µL
Primer Set	6.0 µL

Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! This kit has been optimized for a 15-µL PCR reaction volume to overcome the PCR inhibition expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

3. Prepare reagents. Thaw the Master Mix and the Primer Set, then vortex for 3 seconds and centrifuge briefly before opening the tubes or bottles.

IMPORTANT! Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8 °C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

4. Pipet the required volumes of components into an appropriately sized polypropylene tube.
5. Vortex the reaction mix for 3 seconds, then centrifuge briefly.

6. Dispense 12 µL of the reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate. The final volume in each well is 15 µL (reaction mix plus Prep-n-Go™ Buffer and sample lysate or positive control).

7. Add samples to the reaction plate:

Well(s)	Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate...	
Test samples	3 µL of sample lysate	
Positive control	• For 25 and 26 cycles	3 µL of DNA Control 007
	• For 27 cycles	2 µL of DNA Control 007
	• For 28 cycles	1 µL of DNA Control 007
Note: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.		

   The final volume in each well is 15 µL (reaction mix plus Prep-n-Go™ Buffer and sample lysate or positive control).

8. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

   IMPORTANT! We recommend adhesive film for plate sealing to provide a consistent seal across all wells and prevent evaporation. Do not use caps, which may not provide a consistent seal across all wells.

   IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block, place a MicroAmp® compression pad (Part no. 4312639) on top of the plate to additionally prevent evaporation during thermal cycling. The Veriti® Thermal Cycler does not require a compression pad.

9. Vortex the reaction mix at medium speed for 3 seconds.

10. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.

11. Amplify the samples in a Veriti® 96-well Thermal Cycler or PCR System 9700 with the silver or gold-plated silver 96-well block as described in "Perform PCR" on page 28.

Store the sample lysate

Cap the sample lysate storage tubes or seal the sample lysate storage plate with MicroAmp® Clear Adhesive Film.

Store the sample lysate as needed:

If you are storing the sample lysate...	Then place at...
<2 weeks	2 to 8°C
>2 weeks	–15 to –25°C

These storage recommendations are preliminary pending the results of ongoing stability studies. The effects of multiple freeze-thaw cycles on the lysate have not been fully evaluated. Therefore, multiple freeze-thaw cycles are not recommended.

Perform PCR

IMPORTANT! The GlobalFiler™ Express Kit is validated for use with the Veriti® 96-well Thermal Cycler Part no. 4375786 NOT the Veriti® 96-Well Fast Thermal Cycler (Part no. 4375305). Please ensure you are using the correct Veriti® Thermal Cycler model.

1. Program the thermal cycling conditions.

   IMPORTANT! When using the GeneAmp PCR System 9700, select the **Max** ramping mode. When using the Veriti® Thermal Cycler, select the **100%** ramping rate. DO NOT use 9600 emulation mode.

Initial incubation step	Optimum cycle number†		Final extension	Final hold
	Denature	Anneal/ Extend		
HOLD	CYCLE		HOLD	HOLD
95°C 1 min	94°C 3 sec	60°C 30 sec	60°C 8 min	4°C ∞

† Determine the optimum cycle number for your laboratory according to the instructions on page 19.

2. Load the plate into the thermal cycler and close the heated cover.
3. Start the run.
4. On completion of the run, store the amplified DNA.

If you are storing the DNA...	Then place at...
<2 weeks	2 to 8°C
>2 weeks	–15 to –25°C

IMPORTANT! Protect the amplified products from light.

3 Perform Electrophoresis

Allelic ladder requirements

To accurately genotype samples, you must run an allelic ladder sample along with the unknown samples.

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder(s)
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500xL	1 per injection	24 samples	23 samples + 1 allelic ladder
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130*xl*	1 per injection	16 samples	15 samples + 1 allelic ladder
3730	3 per injection	48 samples	15 samples + 1 allelic ladder

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between both single- and multiple-capillary runs (with larger size variations seen between samples injected in multiple-capillary runs). We recommend the above frequency of allelic ladder injections, which should account for normal variation in run speed. However, during internal validation studies, verify the required allelic ladder injection frequency to ensure accurate genotyping of all samples in your laboratory environment.

It is critical to genotype using an allelic ladder run under the same conditions as the samples, because size values obtained for the same sample can differ between instrument platforms because of different polymer matrices and electrophoretic conditions.

Section 3.1 3500/3500xL instruments

Set up the 3500/3500xL instruments for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 69 lists the required materials not supplied with this kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Electrophoresis software setup and reference documents

The following table lists data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, refer to the documents listed in the table.

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions (installed with the HID Updater)	References
3500†	Windows Vista ®	3500 Data Collection Software v1	HID Updater 3500 DC v2.0 (Part no. 4480670)	• Plate templates: 6dye_36_POP4 • Assays: GF+Norm_POP4 and GF_POP4 which contain instrument protocol HID36_POP4_J6_NT3200 with the following conditions: – Run module: HID36_POP4 – Injection conditions: 1.2 kV/ 16 sec – Run conditions: 13 kV/1550 sec – Dye Set J6	*3500/3500xL Genetic Analyzer User Guide* (Pub. no. 4401661) *HID Updater 3500 Data Collection Software v2 Release Notes*
3500xL†				• Plate templates: 6dye_36_POP4_xl • Assays: GF+Norm_POP4_xl and GF_POP4_xl which contain instrument protocol HID36_POP4xl_J6_NT3200 with the following conditions: – Run module: HID36_POP4 – Injection conditions: 1.2 kV/ 24 sec – Run conditions: 13 kV/1550 sec – Dye Set J6	

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions (installed with the HID Updater)	References
3500† 3500xL†	Windows® 7	3500 Data Collection Software v2	HID Updater 3500 DC v2.0 (Part no. 4480670)	Same as 3500 Data Collection Software v1 listed above	*3500/3500xL Genetic Analyzer User Guide* (Pub. no. 4476988) *HID Updater 3500 Data Collection Software v2 Release Notes*

† We conducted validation studies for the GlobalFiler™ Express Kit using the 3130*xl*, 3500, or 3500xL configurations.

Obtain and run the HID Updater

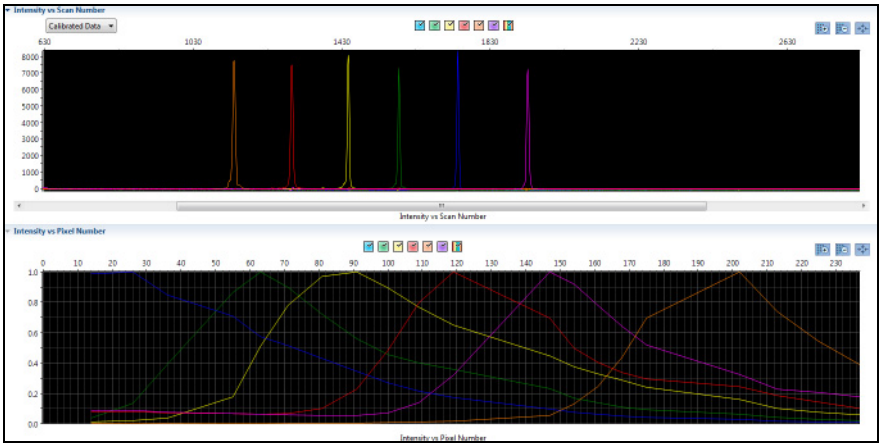
You can run 6-dye samples on 3500 Data Collection Software v1 or v2. Before running on either system for the first time, run the HID Updater 3500 DC v2.0 (Part no. 4480670). The HID Updater installs the plate templates, assays, and instrument protocols needed to run GlobalFiler™ Express Kit samples. For more information, refer to the release notes provided with the Updater.

Note: If you have a new instrument installed by a Life Technologies representative, the updater may have been run during installation.

1. Obtain the HID Updater 3500 DC v2.0 from **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ **GeneMapper® *ID-X* Software**.
2. Exit the 3500/3500xL Data Collection Software.
3. Load the HID Updater Installer CD on the instrument computer.
4. Double click the **HID_Updater_3500_DC_SW_2**.0.exe file on the CD.
5. When installation is complete, restart the computer.

Perform spectral calibration

Perform a spectral calibration using the DS-36 Matrix Standard (J6 Dye Set) (Part no. 4425042). The following figure is an example of a passing 6-dye spectral calibration.



Prepare samples for electrophoresis on the 3500/3500xL instruments

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and GeneScan™ 600 LIZ® Size Standard v2.0 needed to prepare the samples:

Reagent	Volume per reaction
GeneScan™ 600 LIZ® Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	9.5 µL

Note: Include additional samples in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp® Optical 96-Well Reaction Plate, add:
   - 10 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 10 µL of Hi-Di™ Formamide.
5. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
6. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Place the sample tray on the autosampler.
9. Start the electrophoresis run.

Section 3.2 3130/3130*xl* instruments

Set up the 3130/3130*xl* instruments for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 69 lists the required materials not supplied with this kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Electrophoresis software setup and reference documents

The following table lists data collection software and the run modules that can be used to analyze PCR products generated by this kit. For details on the procedures, refer to the documents listed in the table.

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Run modules and conditions	References
3130	Windows® 7	Data Collection Software v4	3130/3730 DC v4 6-Dye Module v1 (contact Life Technologies)	• HIDFragmentAnalysis36_POP4_1 Injection conditions: 3 kV/5 sec Run conditions: 15 kV/1500 sec • Dye Set J6	*Applied Biosystems 3130 Series Data Collection Software v4 Getting Started Guide* (Pub. no. 4477796)
3130*xl*†				• HIDFragmentAnalysis36_POP4_1 Injection conditions: 3 kV/10 sec Run conditions: 15 kV/1500 sec • Dye Set J6	

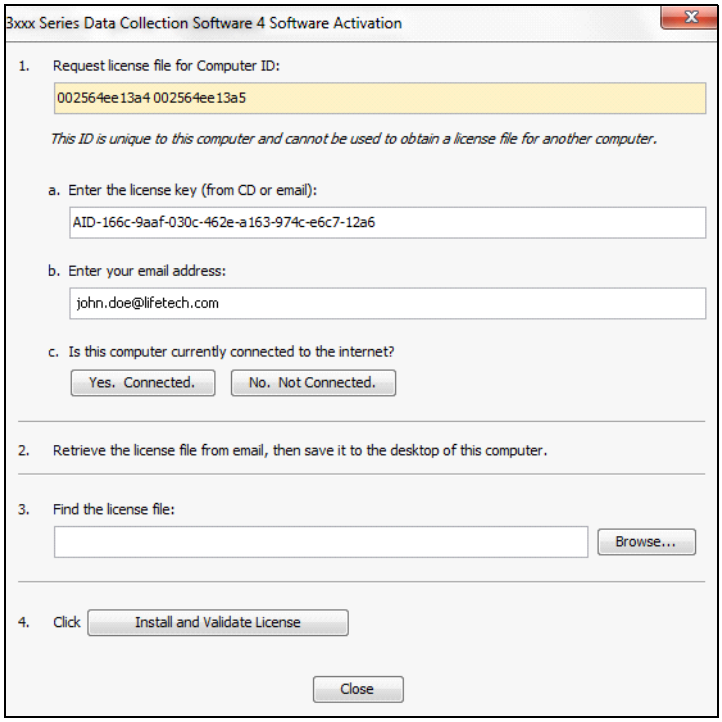
† We conducted validation studies for the GlobalFiler™ Express Kit using the 3130*xl*, 3500, or 3500xL configurations.

Obtain and activate the 6-dye license for the instrument

1. Confirm that you are running Data Collection Software v4 (**Help ▸ About**).
2. Obtain a 3130 DC v4 6-Dye Module v1 License key. Contact Life Technologies for information.
3. Ensure that all network cards in the computer are enabled.

IMPORTANT! You can run the 3130 Series Data Collection Software v4 using only the network cards enabled when you activate the software license. For example, if you activate the software when your wireless network card is disabled, you will not be able to run the software when the wireless network card is enabled.

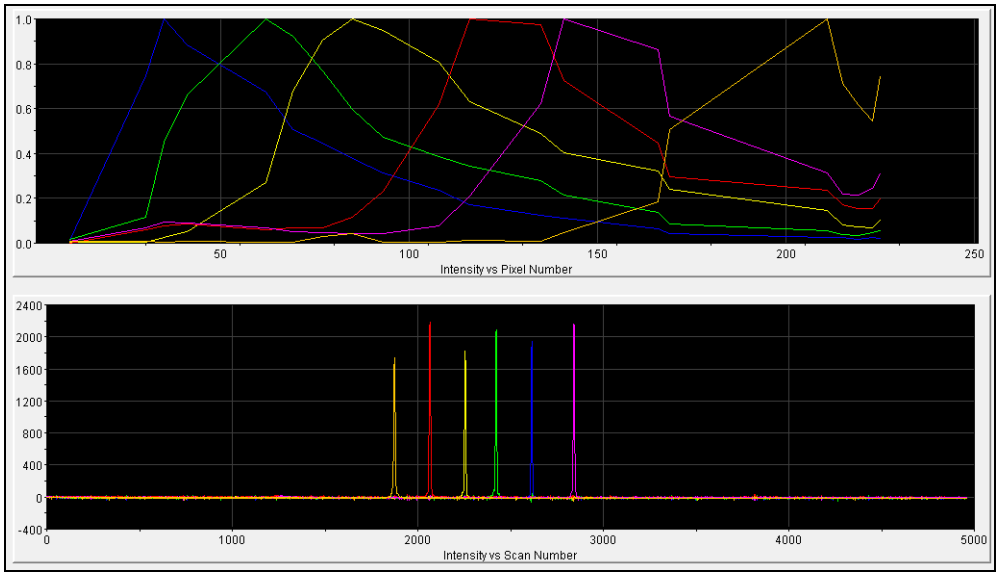
4. Select **Tools ▸ License Manager** to display the Software Activation dialog box.



5. Request the software license file by performing steps **1a**, **1b**, and **1c** as listed on the activation screen. The license file will be emailed to you.
6. Obtain the software license file from your email.
7. Make a copy of the software license file and keep in a safe location.
8. Copy the software license file to the desktop of the Data Collection Software v4 computer.
9. If the Software Activation dialog box has closed, select **Tools ▸ License Manager**.
10. Click **Browse**, then navigate to the software license file saved on your computer.
11. Click **Install and Validate License**. A message is displayed when the license is installed and validated.
12. Click **Close**.

Perform spectral calibration

Perform a spectral calibration using the DS-36 Matrix Standard (J6 Dye Set) (Part no. 4425042). The following figure is an example of a passing 6-dye spectral calibration.



Prepare samples for electrophoresis on the 3130/3130*xl* instruments

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and size standard needed to prepare the samples:

Reagent	Volume per reaction
GeneScan™ 600 LIZ® Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	9.5 µL

Note: Include additional samples in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp® Optical 96-Well Reaction Plate, add:
   - 10 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

Note: For blank wells, add 10 µL of Hi-Di™ Formamide.

5. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
6. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Prepare the plate assembly on the autosampler.
9. Start the electrophoresis run.

Section 3.3 3730 instrument

Set Up the 3730 instrument for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 69 lists the required materials not supplied with the GlobalFiler™ Express Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Electrophoresis software setup and reference documents

The following table lists data collection software and the run modules that you can use to analyze GlobalFiler™ Express Kit PCR products. For details on the procedures, refer to the documents listed in the table.

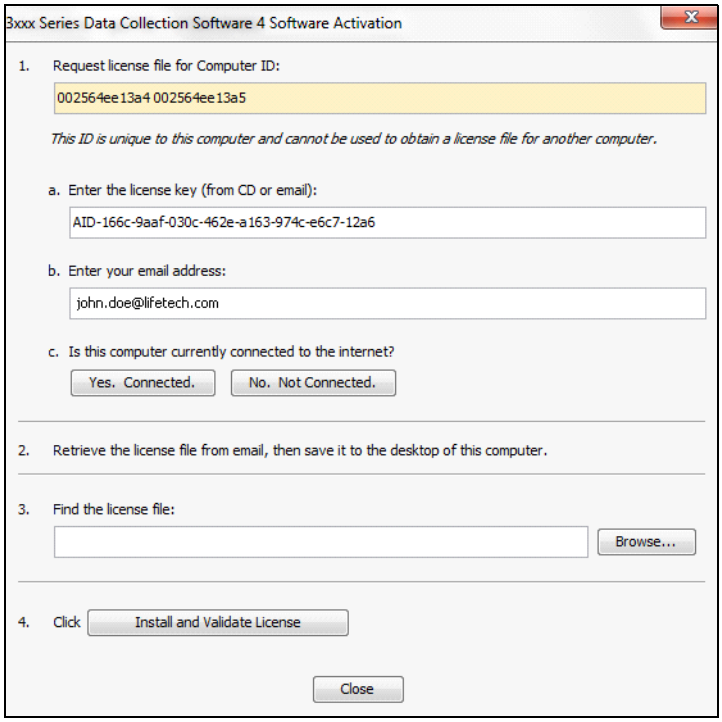
DNA Analyzer	Operating System	Data Collection Software	Additional software	Run module	References
3730	Windows® 7	Data Collection Software v4	3130/3730 DC v4 6-Dye Module v1 (contact Life Technologies)	• GeneMapper36_POP7_1 Injection conditions: 2 kv/10 sec Run conditions: 15 kV/1200 sec • Dye Set J6	*3730/3730xl DNA Analyzer Getting Started Guide* (Pub. no. 4478016)

Obtain and activate the 6-dye license for the instrument

1. Confirm that you are running Data Collection Software v4 (**Help ▸ About**).
2. Obtain a 3730 DC v4 6-Dye Module v1 License key. Contact Life Technologies for information.
3. Ensure that all network cards in the computer are enabled.

IMPORTANT! You can run the 3130 Series Data Collection Software v4 using only the network cards enabled when you activate the software license. For example, if you activate the software when your wireless network card is disabled, you will not be able to run the software when the wireless network card is enabled.

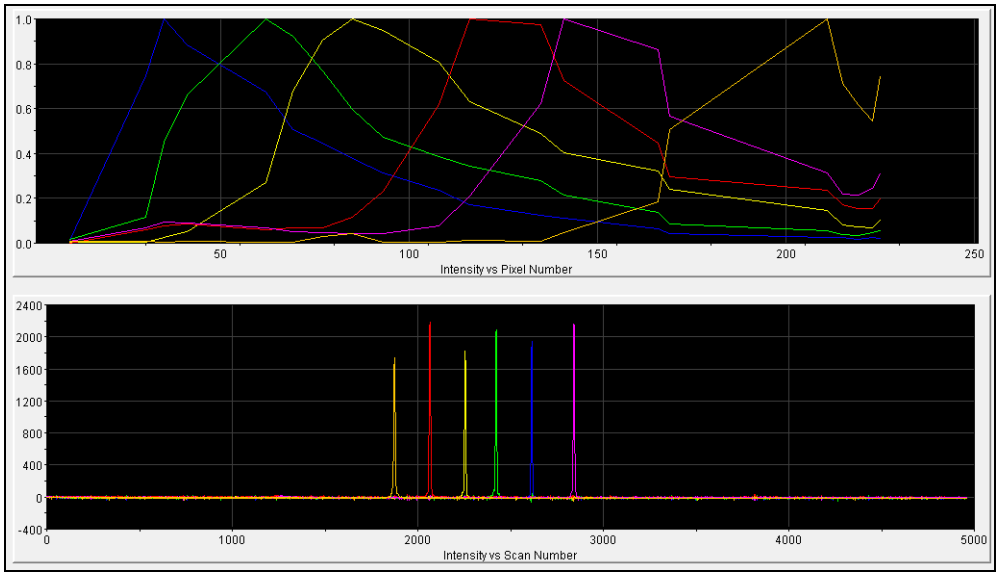
4. Select **Tools ▸ License Manager** to display the Software Activation dialog box.



5. Request the software license file by performing steps **1a**, **1b**, and **1c** as listed on the activation screen. The license file will be emailed to you.
6. Obtain the software license file from your email.
7. Make a copy of the software license file and keep in a safe location.
8. Copy the software license file to the desktop of the Data Collection Software v4 computer.
9. If the Software Activation dialog box has closed, select **Tools ▸ License Manager**.
10. Click **Browse**, then navigate to the software license file saved on your computer.
11. Click **Install and Validate License**. A message is displayed when the license is installed and validated.
12. Click **Close**.

Perform spectral calibration

Perform a spectral calibration using the DS-36 Matrix Standard (J6 Dye Set) (Part no. 4425042). The following figure is an example of a passing 6-dye spectral calibration.



Prepare samples for electrophoresis on the 3730 instrument

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and size standard needed to prepare the samples:

Reagent	Volume per reaction
GeneScan™ 600 LIZ® Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	9.5 µL

Note: Include additional samples in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp® Optical 96-Well Reaction Plate, add:
   - 9 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 10 µL of Hi-Di™ Formamide.

5. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
6. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Place the sample tray on the autosampler.
9. Start the electrophoresis run.

4 Analyze Data

Overview of GeneMapper® *ID-X* Software

GeneMapper® *ID-X* Software is an automated genotyping software for forensic casework, databasing, and paternity data analysis.

GeneMapper® *ID-X* Software v1.4 or higher analyzes 4-dye, 5-dye, and 6-dye data and is required to correctly analyze data generated using the GlobalFiler™ Express Kit.

After electrophoresis, the data collection software stores information for each sample in a .fsa or .hid file. Using GeneMapper® *ID-X* Software v1.4 or higher you can then analyze and interpret the data from the .fsa or .hid files.

IMPORTANT! Because of the small amplicon sizes generated by the GlobalFiler™ Express Kit, the 3rd Order Least Squares Sizing algorithm has been validated for analyzing GlobalFiler™ Express Kit data. For information, see "Create an analysis method" on page 51.

Instruments

Refer to "Instrument and software overview" on page 16 for a list of compatible instruments.

Before you start

When using GeneMapper® *ID-X* Software v1.4 or higher to perform human identification (HID) analysis with GlobalFiler™ Express Kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Your laboratory can use multiple ladder samples in an analysis, provided individual laboratories conduct the appropriate validation studies.

  For multiple ladder samples, the GeneMapper® *ID-X* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.

  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.

- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples to ensure proper allele calling.
- Alleles that are not in the GlobalFiler™ Express Kit Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, the sample result needs to be verified according to the laboratory's protocol.

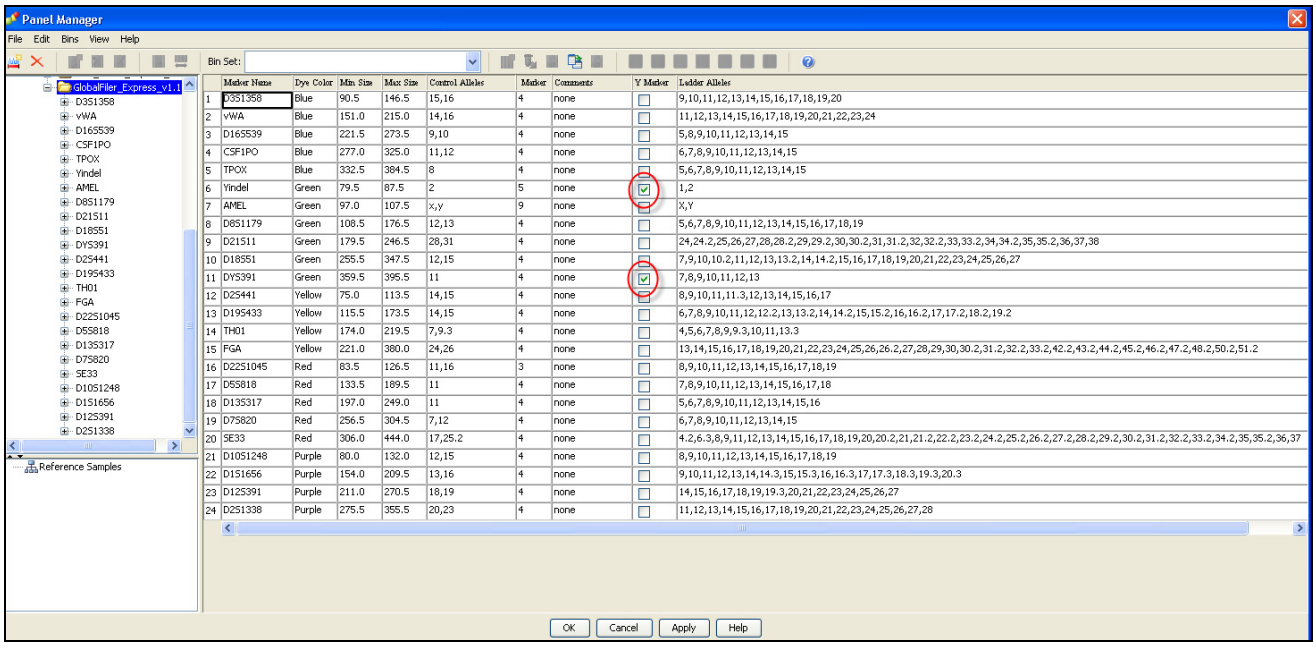
New features to support GlobalFiler™ Express Kit data analysis

GeneMapper® *ID-X* Software v1.4 includes the following new features and updates to support GlobalFiler™ Express Kit data analysis. Refer to the *GeneMapper® ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. no. 4477684) for information on other new features of the software.

- Analyzes data generated with the GlobalFiler™ Express Kit and the J6 dye set.
- The following analysis files are automatically installed and imported into the software:
  - Two new GeneScan™ 600 LIZ® size standards: GS600_LIZ+Normalization_(60-460) and GS600_LIZ_(60-460)
  - Panels, bins, table settings, and plot settings updated for the GlobalFiler™ Express Kit
  - CODIS Marker Properties file
- The following analysis files are automatically installed must be imported into the software before use (see "Import panels, bins, and marker stutter" on page 47): stutter settings.
- Y-marker analysis. In addition to the ability to analyze autosomal STR and Y-STR data separately, GeneMapper® *ID-X* Software v1.4 can analyze autosomal and Y-STR markers in one multiplexed sample. A new marker-level PQV, Amelogenin Cross Check (ACC) has been added and the Allele Number (AN)

PQV has been enhanced to evaluate the quality of Y markers designated for analysis in the Panel Manager. Y markers designated in the Panel Manager are excluded from the Mixture Analysis and Statistical Calculations performed in the Mixture Analysis Tool.

By default, the software designates two loci in the GlobalFiler™ Express panel, DYS391 and the Y Indel, as Y markers, and processes all other loci as autosomal.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker	Comments	Y Marker	Ladder Alleles
1	D3S1358	Blue	90.5	146.5	15,16	4	none	☐	9,10,11,12,13,14,15,16,17,18,19,20
2	vWA	Blue	151.0	215.0	14,16	4	none	☐	11,12,13,14,15,16,17,18,19,20,21,22,23,24
3	D16S539	Blue	221.5	273.5	9,10	4	none	☐	5,8,9,10,11,12,13,14,15
4	CSF1PO	Blue	277.0	325.0	11,12	4	none	☐	6,7,8,9,10,11,12,13,14,15
5	TPOX	Blue	332.5	384.5	8	4	none	☐	5,6,7,8,9,10,11,12,13,14,15
6	Yindel	Green	79.5	87.5	2	5	none	☑	1,2
7	AMEL	Green	97.0	107.5	X,Y	9	none	☐	X,Y
8	D8S1179	Green	108.5	176.5	12,13	4	none	☐	5,6,7,8,9,10,11,12,13,14,15,16,17,18,19
9	D21S11	Green	179.5	246.5	28,31	4	none	☐	24,24.2,25,26,27,28,28.2,29,29.2,30,30.2,31,31.2,32,32.2,33,33.2,34,34.2,35,35.2,36,37,38
10	D18S51	Green	255.5	347.5	12,15	4	none	☐	7,9,10,10.2,11,12,13,13.2,14,14.2,15,16,17,18,19,20,21,22,23,24,25,26,27
11	DYS391	Green	359.5	395.5	11	4	none	☑	7,8,9,10,11,12,13
12	D2S441	Yellow	75.0	113.5	14,15	4	none	☐	8,9,10,11,11.3,12,13,14,15,16,17
13	D19S433	Yellow	115.5	173.5	14,15	4	none	☐	6,7,8,9,10,11,12,12.2,13,13.2,14,14.2,15,15.2,16,16.2,17,17.2,18.2,19.2
14	TH01	Yellow	174.0	219.5	7,9.3	4	none	☐	4,5,6,7,8,9,9.3,10,11,13.3
15	FGA	Yellow	221.0	380.0	24,26	4	none	☐	13,14,15,16,17,18,19,20,21,22,23,24,25,26,26.2,27,28,29,30,30.2,31.2,32.2,33.2,42.2,43.2,44.2,45.2,46.2,47.2,48.2,50.2,51.2
16	D22S1045	Red	83.5	126.5	11,16	3	none	☐	8,9,10,11,12,13,14,15,16,17,18,19
17	D5S818	Red	133.5	189.5	11	4	none	☐	7,8,9,10,11,12,13,14,15,16,17,18
18	D13S317	Red	197.0	249.0	11	4	none	☐	5,6,7,8,9,10,11,12,13,14,15,16
19	D7S820	Red	256.5	304.5	7,12	4	none	☐	6,7,8,9,10,11,12,13,14,15
20	SE33	Red	306.0	444.0	17,25.2	4	none	☐	4.2,6.3,8,9,11,12,13,14,15,16,17,18,19,20,20.2,21,21.2,22.2,23.2,24.2,25.2,26.2,27.2,28.2,29.2,30.2,31.2,32.2,33.2,34.2,35,35.2,36,37
21	D10S1248	Purple	80.0	132.0	12,15	4	none	☐	8,9,10,11,12,13,14,15,16,17,18,19
22	D1S1656	Purple	154.0	209.5	13,16	4	none	☐	9,10,11,12,13,14,14.3,15,15.3,16,16.3,17,17.3,18.3,19.3,20.3
23	D12S391	Purple	211.0	270.5	18,19	4	none	☐	14,15,16,17,18,19,19.3,20,21,22,23,24,25,26,27
24	D2S1338	Purple	275.5	355.5	20,23	4	none	☐	11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28

IMPORTANT! Do not select Amelogenin (AMEL) as a Y marker in Panel Manager. The AN PQV flag for AMEL follows Amelogenin-specific rules, which differ from the rules used for Y markers. For more information, see the *GeneMapper® ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. no. 4477684).

Set up GeneMapper® *ID-X* Software for data analysis

Panel, bin, and stutter file version

The file names shown in this section may differ from the file names you see when you download or import files. If you need help determining the correct files to use, contact your local Life Technologies Human Identification representative, or go to **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ **GeneMapper® *ID-X* Software**.

The instructions and examples in this section refer to the latest version of panel, bin, and stutter file available at the time of publication.

Before using the software for the first time

Before you use GeneMapper® *ID-X* Software v1.4 to analyze data for the first time, you must do the following:

1. Check the version of panel, bin, and stutter files installed with the GeneMapper® *ID-X* Software as explained in "Check panel, bin, and stutter file version" below.
2. Check **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ **GeneMapper® *ID-X* Software** to determine if newer files are available.
3. If updated files are available, download and import the files into the GeneMapper® *ID-X* Software, as explained in "Import panels, bins, and marker stutter" on page 47.

   Note: When downloading new versions of analysis files, refer to the associated Read Me file for details of changes between software file versions. If you have validated previous file versions for data analysis, conduct the appropriate internal verification studies before using new file versions for operational analysis.
4. Create an analysis method, as explained in "Create an analysis method" on page 51.
5. Define custom views of analysis tables.

   A default analysis table for six-dye analysis is provided in the GeneMapper® *ID-X* Software v1.4. Refer to Chapter 1 of the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574) for general information on default table settings.
6. Define custom views of plots.

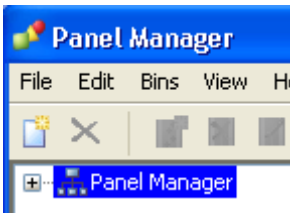
   A default plot for six-dye analysis is provided in the GeneMapper® *ID-X* Software v1.4. Refer to Chapter 1 of the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574) for general information on default plot settings.

Check panel, bin, and stutter file version

1. Start the GeneMapper® *ID-X* Software, then log in with the appropriate user name and password.

   IMPORTANT! For logon instructions, refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574).
2. Select **Tools** ▸ **Panel Manager**.

3. Check the version of files imported into the Panel Manager:



   a. Select **Panel Manager** in the navigation pane.

   b. Expand the Panel Manager folder and any sub-folders to identify the analysis file version already installed for your kit choice.

4. Check the version of files available for import into the Panel Manager:

   a. Select **Panel Manager**, then select **File ▸ Import Panels** to open the Import Panels dialog box.

   b. Navigate to, then open the Panels folder and check the version of panel, bin, and stutter files installed.

5. If newer versions are available on the website, download and import as described below.

Import panels, bins, and marker stutter

Note: The AmpFLSTR v3X panel and bin files are automatically imported into the GeneMapper® *ID-X* Software v1.4 database during installation. The v3X Stutter file is also installed but must be imported before use.

To import the GlobalFiler™ Express Kit panel, bin set, and marker stutter from our web site into the GeneMapper® *ID-X* Software database:

1. Download and open the file containing panels, bins, and marker stutter:

   a. Go to **www.lifetechnologies.com/support ▸ Software, Patches & Updates ▸ GeneMapper® *ID-X* Software**. Download the file **AmpFLSTR Analysis Files v3X**.

   b. Unzip the file.

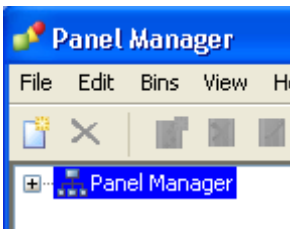
2. Start the GeneMapper® *ID-X* Software, then log in with the appropriate user name and password.

   IMPORTANT! For logon instructions, refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574).

3. Select **Tools ▸ Panel Manager**.

4. Find, then open the folder containing the panels, bins, and marker stutter:

   a. Select **Panel Manager** in the navigation pane.

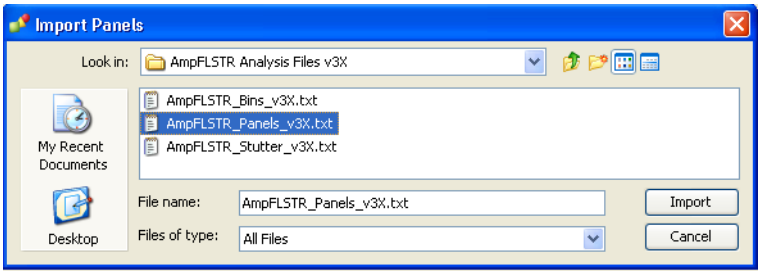


   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.

   c. Navigate to, then open the **AmpFLSTR Analysis Files v3X** folder that you unzipped in step 1 on page 47.

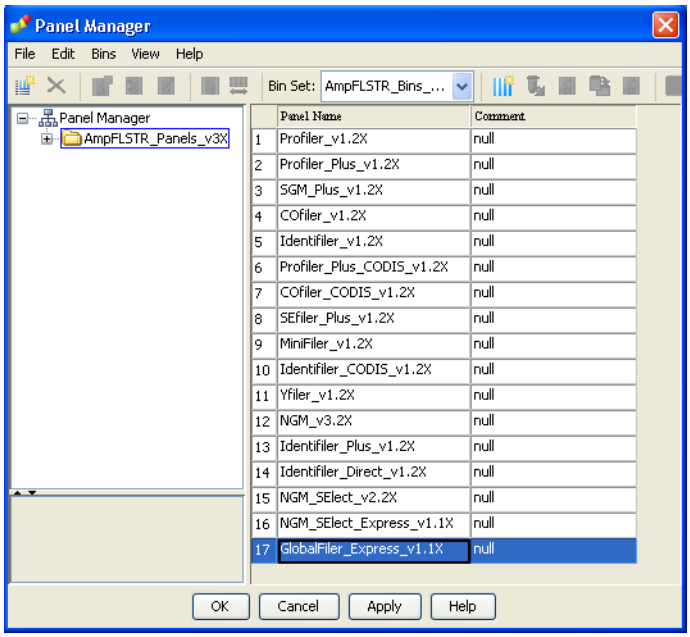
5. Select AmpFLSTR_Panels_v3X (or the version you installed), then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager "AmpFLSTR_Panels_v3X". This folder contains panels for multiple kits and associated markers.



6. Import AmpFLSTR_Bins_v3X.txt:

   a. Select the AmpFLSTR_Panels_v3X folder in the navigation pane.

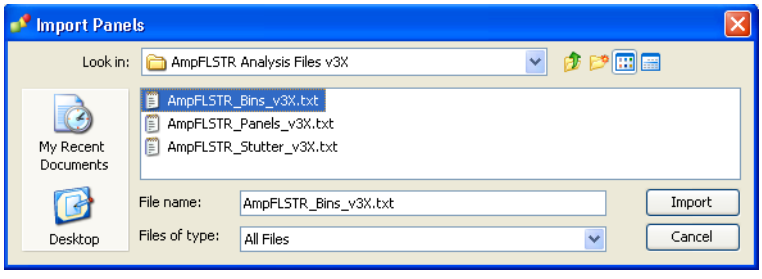


   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

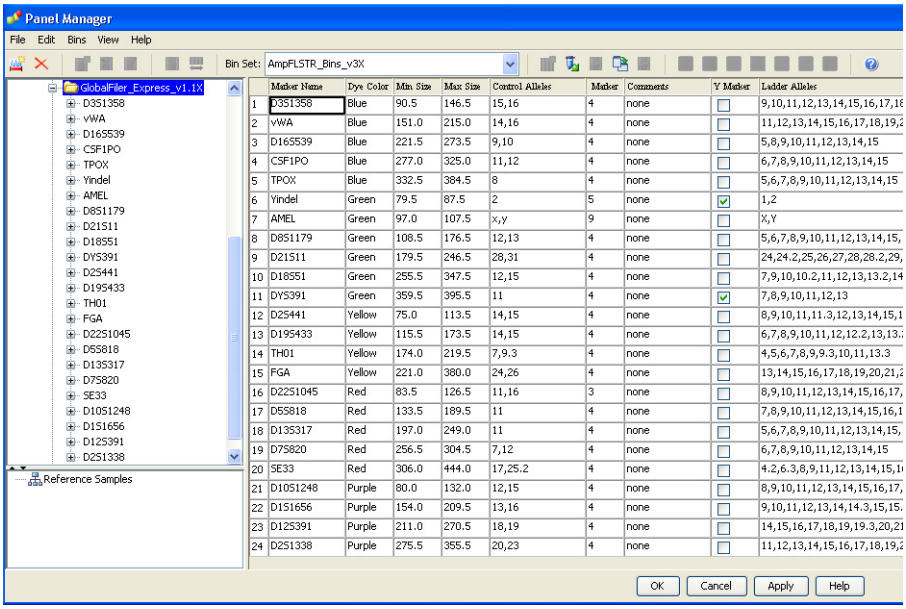
   c. Navigate to, then open the **AmpFLSTR Analysis Files v3X** folder.

   d. Select **AmpFLSTR_Bins_v3X.txt**, then click **Import**.

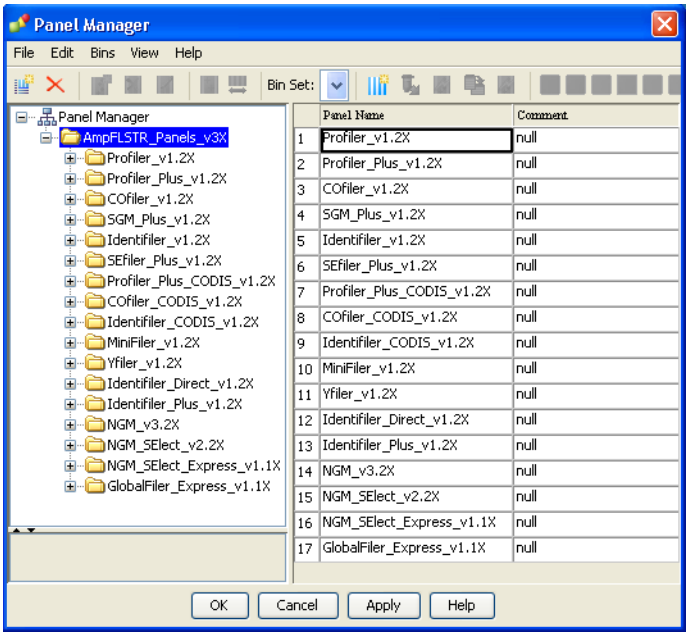
   Note: Importing this file associates the bin set with the panels in the AmpFLSTR_Panels_v3X folder.



7. View the imported panels in the navigation pane:
   a. Double-click the AmpFLSTR_Panels_v3X folder.
   b. Double-click the **GlobalFiler_Express_v1.1X** folder to display the panel information in the right pane.



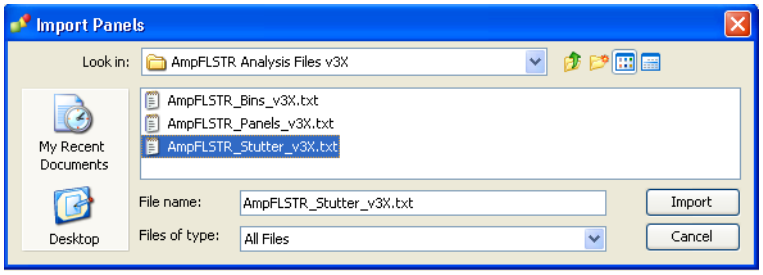
8. Import AmpFLSTR_Stutter_v3X.txt:
   a. Select the AmpFLSTR_Panels_v3X folder in the navigation panel.



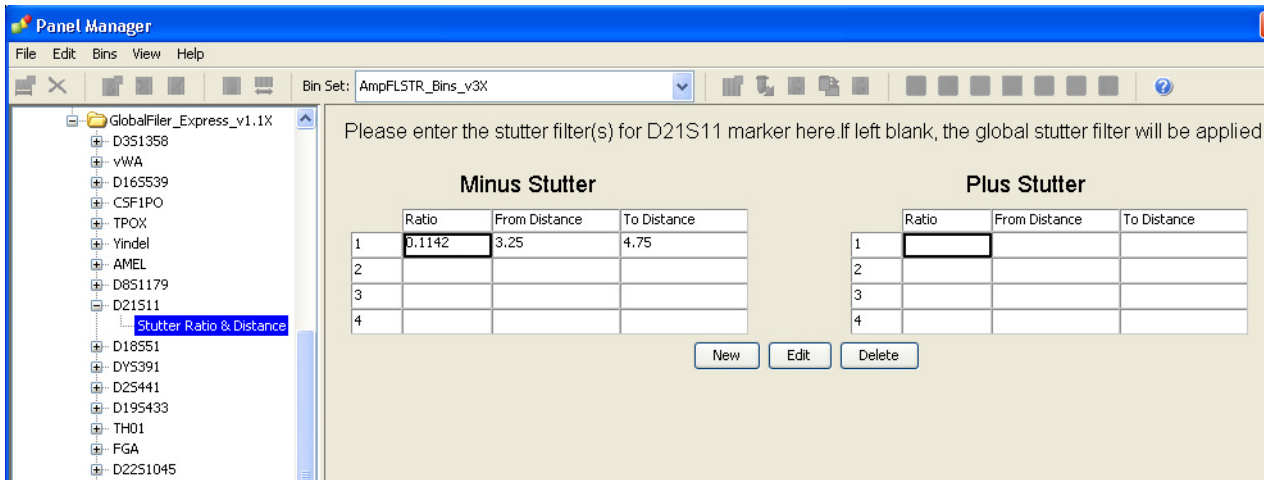
   b. Select File ▸ **Import Marker Stutter** to open the Import Marker Stutter dialog box.
   c. Navigate to, then open the **AmpFLSTR Analysis Files v3X** folder.

d. Select **AmpFLSTR_Stutter_v3X.txt**, then click **Import**.

Note: Importing this file associates the marker stutter ratio with the bin set in the AmpFLSTR_Panels_v3X folder and overwrites any existing stutter ratios associated with the panels and bins in that folder.



9. View the imported marker stutters in the navigation pane:
   a. Double-click the AmpFLSTR_Panels_v3X folder to display its list of kits in the right pane.
   b. Double-click the **GlobalFiler_Express_v1.1X** folder to display its list of markers below it.
   c. Double-click **D21S11**, then click **Stutter Ratio & Distance** to display the Stutter Ratio & Distance view for the marker in the right pane.



10. Click **Apply**, then **OK** to add the GlobalFiler™ Express Kit panel, bin set, and marker stutter to the GeneMapper® *ID-X* Software database.

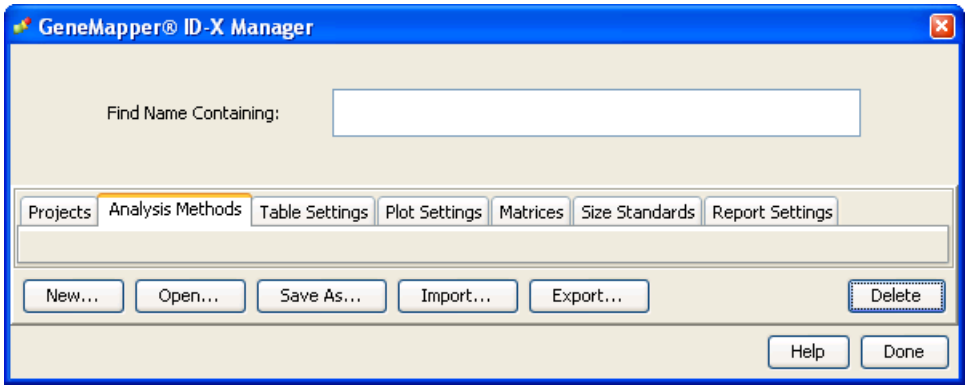
IMPORTANT! If you close the Panel Manager without clicking **Apply**, the panels, bin sets, and marker stutter will not be imported into the GeneMapper® *ID-X* Software database.

Create an analysis method

Use the following procedure to create an analysis method for the GlobalFiler™ Express Kit.

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method created for GeneMapper® *ID-X* version 1.2 is not compatible with earlier versions of GeneMapper® *ID-X* Software or with GeneMapper® *ID* Software version 3.2.1.

1. Select **Tools** ▸ **GeneMapper® ID-X Manager** to open the GeneMapper® *ID-X* Manager.



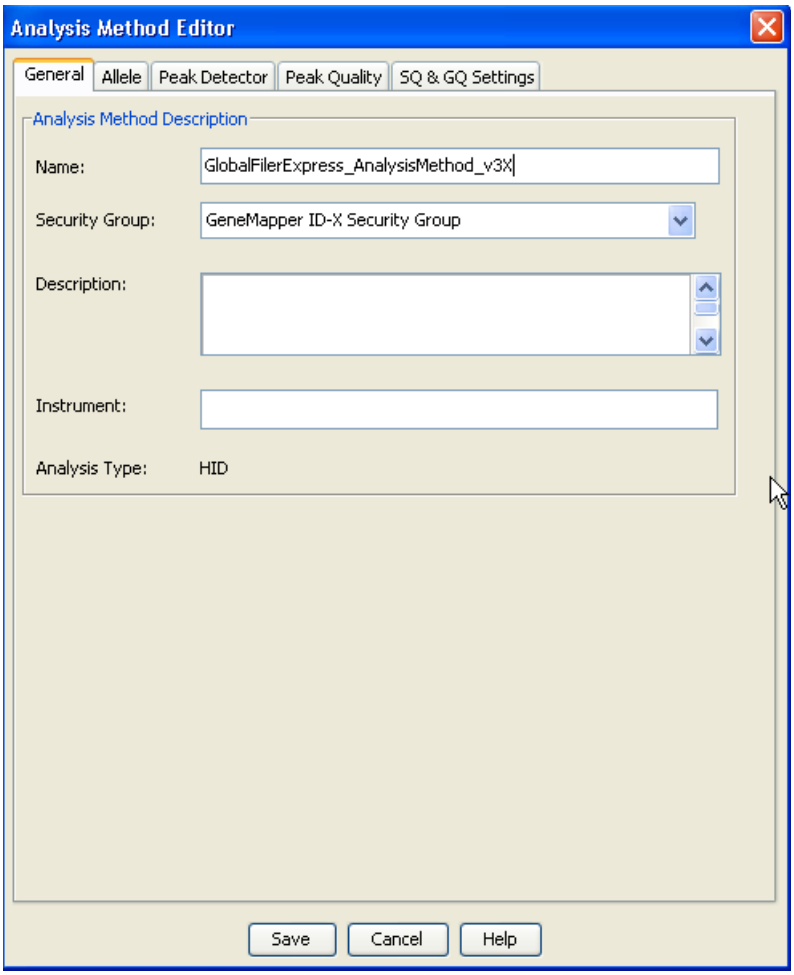
2. Select the **Analysis Methods** tab, then click **New** to open the Analysis Method Editor with the **General** tab selected.

   The figures below show the settings for each tab of the Analysis Method Editor. Configure the Analysis Method Editor tab settings as shown in the figures below, unless the instructions state otherwise.

   Note: The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

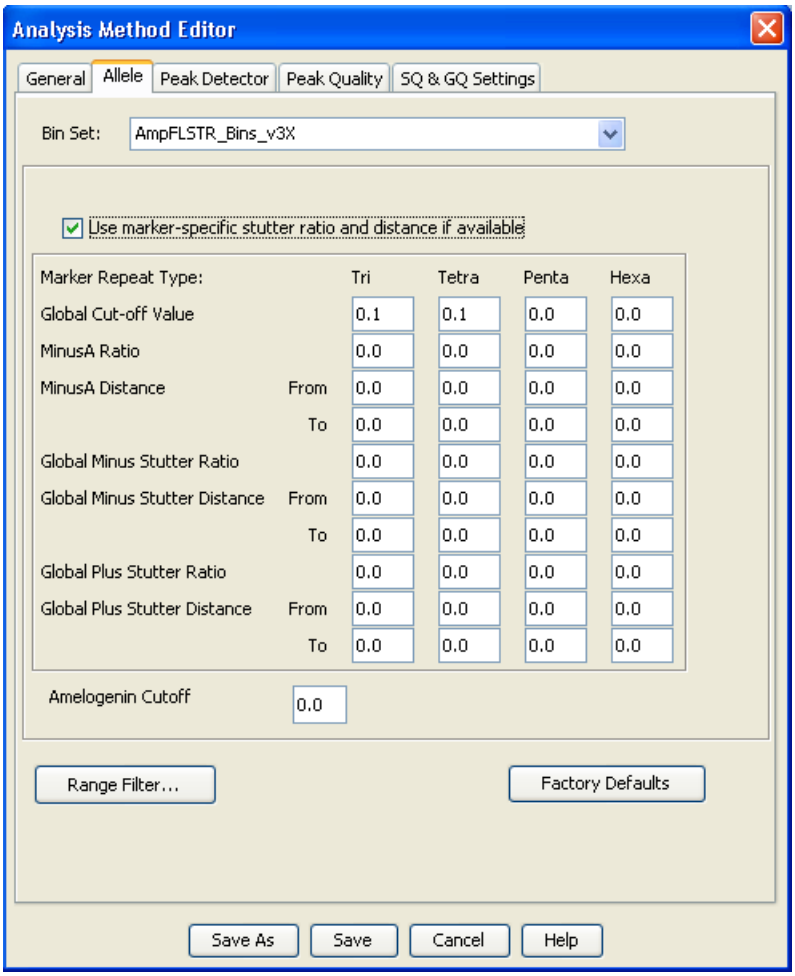
3. After you enter settings in all tabs, click **Save**.

General tab settings



In the Name field, either type the name as shown or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the dropdown list. The Description and Instrument fields are optional.

Allele tab settings



The following settings were used during developmental validation of the GlobalFiler™ Express Kit:

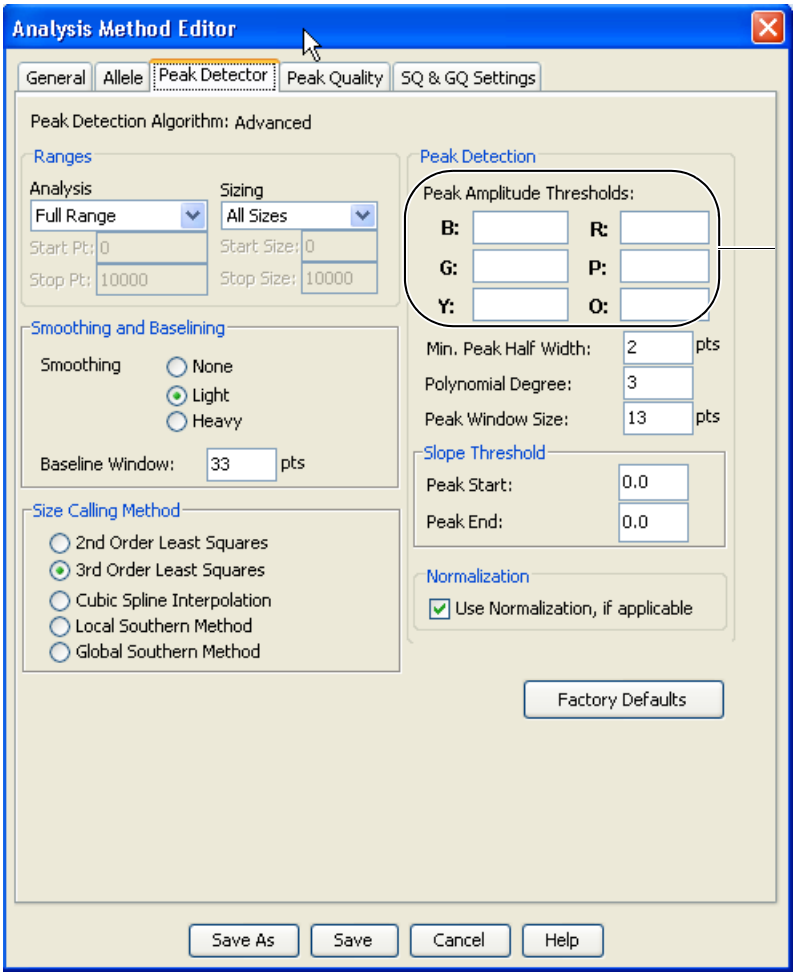
- In the Bin Set field, select the **AmpFLSTR_Bins_v3X** bin set.
- GeneMapper® *ID-X* Software v1.0.1 or higher allows you to specify 4 types of marker repeat motifs: tri, tetra, penta and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the appropriate filter settings. To apply the stutter ratios contained in the AmpFLSTR_Stutter_v3X.txt file, select the "Use marker-specific stutter ratio if available" check box (selected by default).

  Note: Additionally, applying a Global Cut-off Value may reduce the editing required for single-source sample data.

Perform appropriate internal validation studies to determine the appropriate filter setting to use.

Peak Detector tab settings

IMPORTANT! Because of the small amplicon sizes generated by the GlobalFiler™ Express Kit, the 3rd Order Least Squares Sizing algorithm has been validated for analysis of GlobalFiler™ Express Kit data.



Perform internal validation studies to determine settings

IMPORTANT! Perform the appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of GlobalFiler™ Express Kit data.

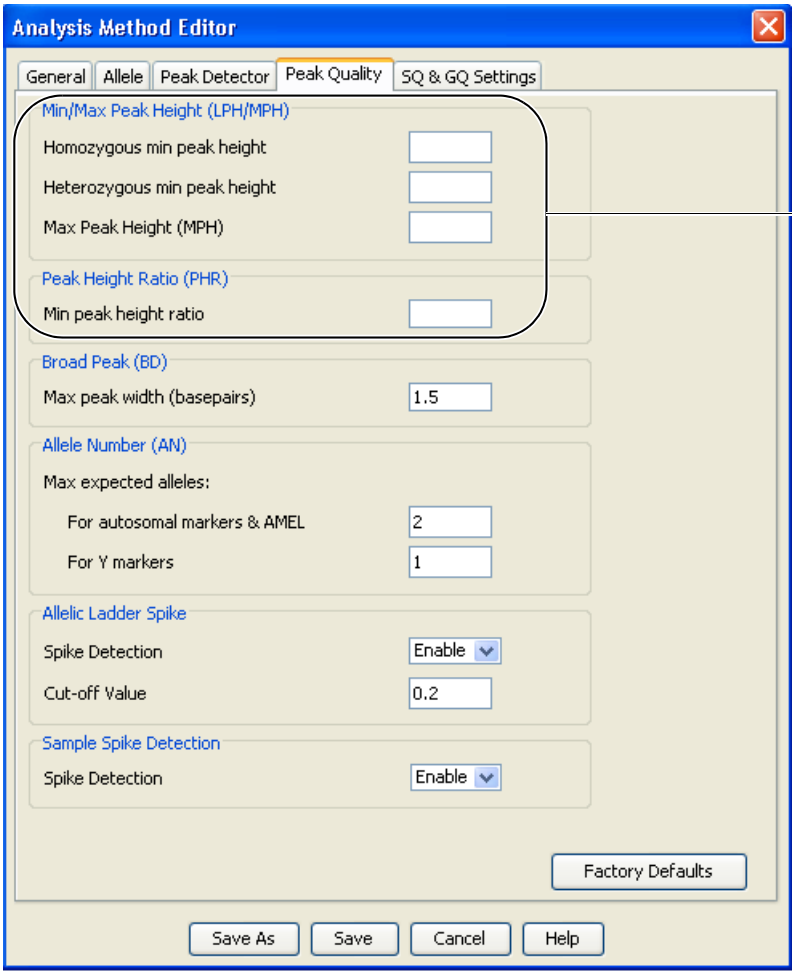
Fields include:

- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper® *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Smoothing – 3730 Genetic Analyzer with POP-7™ polymer only:** With the default Smoothing setting of Light, the D2S441 and D1S1656 markers in some allelic ladder samples did not pass the base-pair spacing quality assessment. The instances of spacing failures were significantly reduced by using the None setting.

  For more information, refer to the GeneMapper® *ID-X* Software Version 1.4 User Bulletin (Pub. no. 4477684 Rev. B), "Known issues: 3730 DNA Analyzer allelic ladder failures".

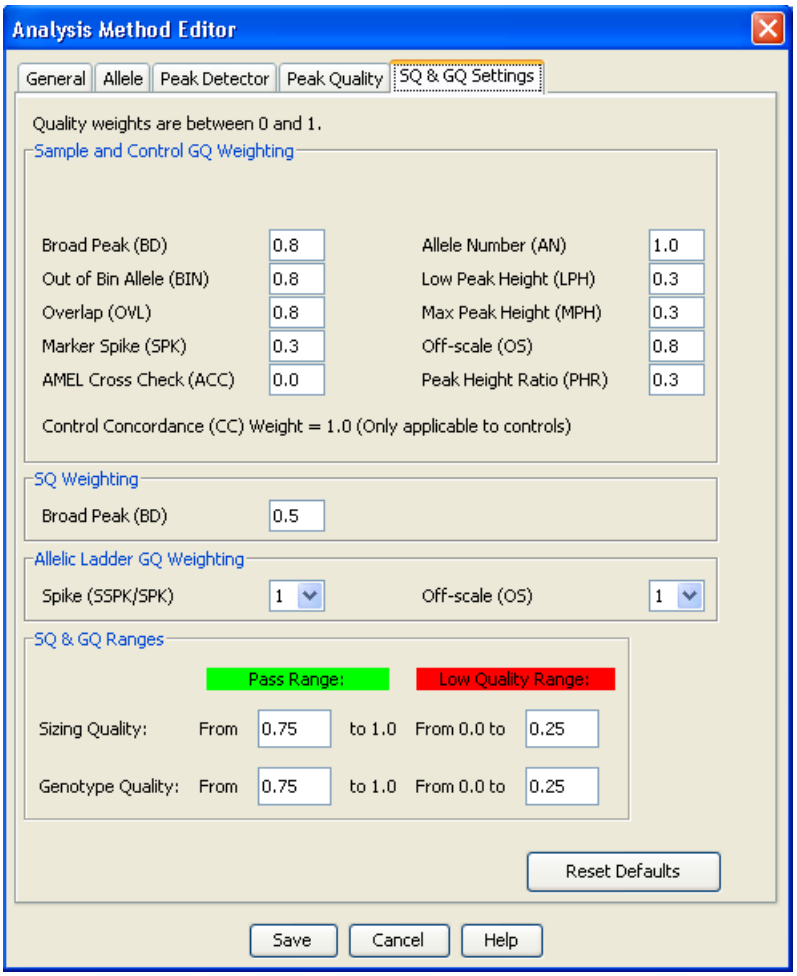
- **Size calling method** – The GlobalFiler™ Express Kit has been validated using the 3rd Order Least Squares sizing method. Select alternative sizing methods only after performing the appropriate internal validation studies.
- **Normalization** – A Normalization checkbox is available on this tab in GeneMapper® *ID-X* Software for use in conjunction with data run on the 3500 Series Genetic Analyzers.

Peak Quality tab settings



IMPORTANT! Perform the appropriate internal validation studies to determine the heterozygous and homozygous minimum peak height thresholds, maximum peak height threshold and the minimum peak height ratio threshold for interpretation of GlobalFiler™ Express Kit data.

SQ & GQ tab settings



IMPORTANT! The values shown are the software defaults and are the values we used during developmental validation. Perform appropriate internal validation studies to determine the appropriate values to use.

Set the ACC GQ Weighting according to your laboratory's use of the ACC PQV. For example, set the ACC GQ Weighting to 0.3 or higher to flag samples in which the Amelogenin result is anything other than X, X or X, Y, or does not agree with the results for the DYS391 or the Y indel markers.

Create a size standard

The size standard for the GeneScan™ 600 LIZ® Size Standard v2.0 contains the following peaks:

GeneScan™ 600 LIZ® Size Standard v2.0
60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, and 460

Note: The GS600_LIZ_(60-460) size standard definition provided with GeneMapper® *ID-X* Software v1.4 has been validated for use with 3130/3130*xl*, 3500/3500xL, and 3730 instruments. However, when running GlobalFiler™ Express Kit samples on the 3730 instrument with POP-7™ polymer, the 60 bp size-standard peak may occasionally be obscured by the primer peak.

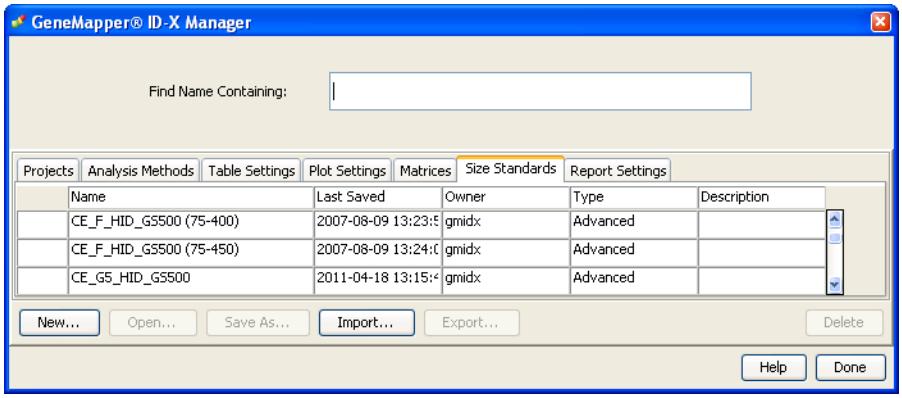
This issue can be addressed by either of the following steps:

- Re-inject samples that fail to recognize the 60 base-pair peak.
- Use the 80 to 460 bp size-standard definition after performing appropriate validation studies (as a general rule, the 60 base-pair peak is not required for accurate fragment sizing using the 3rd Order Least Squares sizing method).

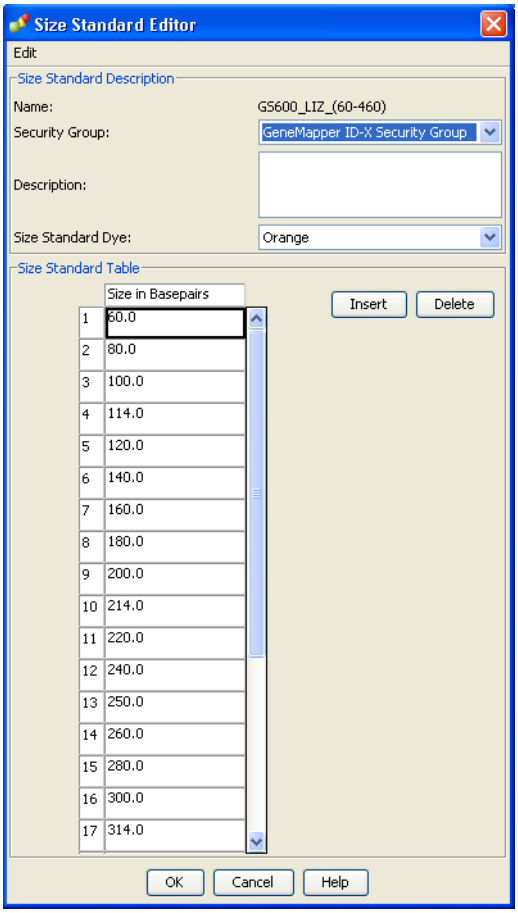
For more information, refer to the GeneMapper® *ID-X* Software Version 1.4 User Bulletin (Pub. no. 4477684 Rev. B), "Known issues: 3730 DNA Analyzer sizing failures".

Use the following procedure to create the size standard definition file:

1. Select **Tools ▸ GeneMapper® *ID-X* Manager** to open the GeneMapper® *ID-X* Manager.
2. Select the **Size Standards** tab, then click **New**.



3. Complete the Name field as shown below or with a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the drop-down list. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified on page 56.



Analyze and edit sample files with GeneMapper® *ID-X* Software

1. In the Project window, select **Edit ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project.

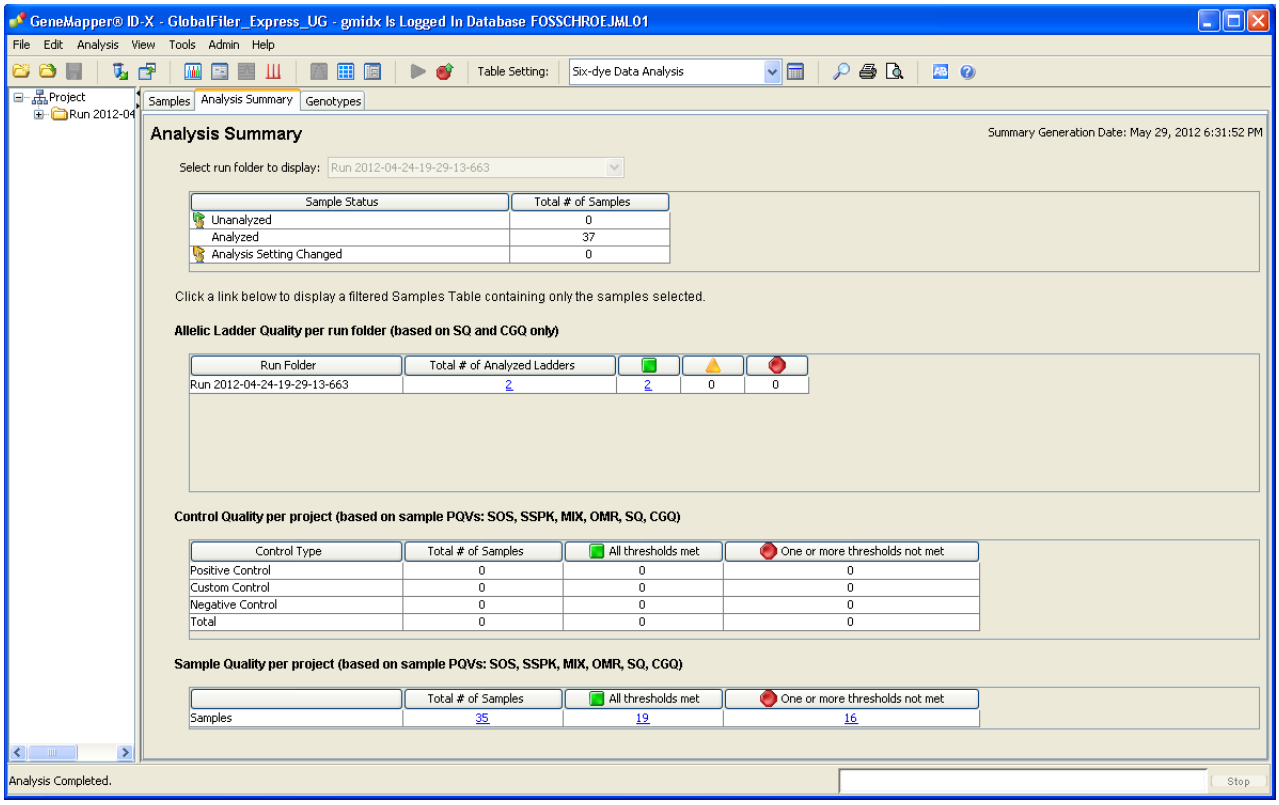
Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	GlobalFilerExpress_AnalysisMethod_v3X (or the name of the analysis method you created)
Panel	GlobalFiler_Express_v1.1X
Size Standard	GS600_LIZ_(60-460)† (or the name of the size standard you created)

† The GlobalFiler™ Express Kit was originally validated using the GeneScan™ 600 LIZ® Size Standard v2.0. If you use the a different size standard, perform the appropriate internal validation studies to support the use of this size standard with the GlobalFiler™ Express Kit.

Note: For more information about how the Size Caller works, refer to the *GeneScan™ Analysis Software for the Windows® NT Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (Pub. no. 4335617).

3. Click (**Analyze**), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis as a completion bar extending to the right with the percentage indicated.
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The Analysis Summary tab is displayed and the Genotypes tab becomes available upon completion of the analysis.

   Analysis summary window after analysis



Examine and edit a project

You can display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data. These procedures start with the Analysis Summary tab of the Project window (assuming the analysis is complete).

For more information

For more information, refer to:

- *GeneMapper® ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. no. 4477684)
- *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574)
- *GeneMapper® ID-X Software Version 1.0 Quick Reference Guide* (Pub. no. 4375670)

- *GeneMapper® ID-X Software Version 1.0 Reference Guide* (Pub. no. 4375671)
- *GeneMapper® ID-X Software Version 1.1(Mixture Analysis) Getting Started Guide* (Pub. no. 4396773)
- *GeneMapper® ID-X Software Version 1.2 Reference Guide* (Pub. no. 4426481)
- *GeneMapper® ID-X Software Version 1.2 Quick Reference Guide* (Pub. no. 4426482)

5 Experiments and Results

TO BE PROVIDED IN NEXT REVISION

A Troubleshooting

Follow the actions recommended in this appendix to troubleshoot problems that occur during analysis.

Table 3 Troubleshooting

Observation	Possible causes	Recommended actions
Faint or no signal from both the DNA Control 007 and the DNA test samples at all loci	Incorrect volume or absence of Master Mix or Primer Set	Repeat amplification.
	No activation of DNA Polymerase	Repeat amplification, making sure to hold reactions initially at 95°C for 1 minute.
	Master Mix not vortexed thoroughly before aliquoting	Vortex the Master Mix thoroughly.
	Primer Set exposed to too much light	Store the Primer Set protected from light.
	Evaporation.	Ensure that the plate is properly sealed with film and that you used a compression pad with the 9700 thermal cycler (a compression pad is not needed with the Veriti® thermal cycler).
	PCR System malfunction	Refer to the thermal cycler user's manual and check instrument calibration.
	Use of incorrect thermal cycling parameters	Check the protocol for correct thermal cycling parameters.
	MicroAmp® Base used with tray/ retainer set and tubes in GeneAmp® 9700	Remove MicroAmp Base from tray/retainer set and repeat test.
	Insufficient PCR product electrokinetically injected	Prepare PCR product as described in Chapter 3, "Perform Electrophoresis" on page 29.
	Degraded formamide	Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.
	Sample punch location was not optimal	For blood samples on treated paper, punch in the center of the blood stain. For buccal samples on treated paper, punch in the center of the buccal transfer or punch in the optimal spot based on past experiences. For buccal samples collected with the Bode Buccal DNA Collector™, punch from near the tip of the collector.
	Insufficient lysis of the swab head	Ensure swab heads are incubated for 20 minutes in 400 µL Prep-N-Go™ buffer.

Observation	Possible causes	Recommended actions
More than expected number of alleles present at a locus	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Amplification of stutter product (–1 repeat unit position)	See "Experiments and Results" on page 63.
	Incomplete 3´ A base addition (n-1 nt position)	See "Experiments and Results" on page 63. Be sure to include the final extension step of 60°C for 5 minutes in the PCR.
	Signal exceeds dynamic range of instrument (off-scale data)	Ensure cycle number is optimized according to instructions on page 19. Repeat PCR amplification using fewer PCR cycles or use your laboratory's SOP to analyze off-scale data.
	Poor spectral separation (bad matrix)	Follow the steps for creating a spectral file.
		Confirm that Filter Set J6 modules are installed and used for analysis.
	Contamination carried over from the disc punching tool	Clean the disc punching tool thoroughly. If necessary, include a blank punch step in between the sample punches.
	Incomplete denaturation of double stranded DNA	Use recommended amount of Hi-Di™ Formamide and perform heat denaturation step according to the instructions in Chapter 3, "Perform Electrophoresis".
Some but not all loci visible on electropherogram of DNA Test Samples	Disc size used in the amplification reaction was greater than 1.2 mm	Repeat amplification using a use 1.2 mm punch size.
	Insufficient volume of swab lysate added to the reaction	Repeat amplification using the recommended lysate input volume.
	Less than 15 µL of PCR reaction volume was used	Repeat amplification using the recommended PCR reaction volume of 15 µL.
STR profiles contain many off-scale alleles	PCR cycle number was too high	Perform sensitivity experiment (page 19) to determine the optimal PCR cycle number based on the sample type.
	For blood samples: Too much liquid blood was spotted onto paper substrate	Spot <100 µL of liquid blood per sample area.
Data collected on the 3730 instrument with POP-7™ polymer fails sizing	The 60 bp size-standard peak is occasionally obscured by the primer peak	• Re-inject samples that fail to recognize the 60 base-pair peak. • Use the 80 to 460 bp size-standard definition after performing appropriate validation studies (as a general rule, the 60 base-pair peak is not required for accurate fragment sizing using the 3rd Order Least Squares sizing method). For more information, refer to the *GeneMapper® ID-X Software Version 1.4 User Bulletin* (Pub. no. 4477684 Rev. B), "Known issues: 3730 DNA Analyzer sizing failures".

Observation	Possible causes	Recommended actions
Data collected on the 3730 instrument with POP-7™ polymer: the D2S441 and D1S1656 markers in some allelic ladder samples did not pass the base-pair spacing quality assessment	Data was analyzed using the Light setting for Smoothing	Use the None setting for smoothing after performing appropriate validation studies. For more information, refer to the *GeneMapper® ID-X Software Version 1.4 User Bulletin* (Pub. no. 4477684 Rev. B), "Known issues: 3730 DNA Analyzer allelic ladder failures".

B Ordering Information

Equipment and materials not included

Table 4 Equipment

Equipment	Source
3500/3500xL Genetic Analyzer	Contact your local Life Technologies sales representative
Veriti® 96-Well Thermal Cycler	4375786
GeneAmp® PCR System 9700 with the Silver 96-Well Block	N8050001
GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block	4314878
Silver 96-Well Sample Block	N8050251
Gold-plated Silver 96-Well Sample Block	4314443
Tabletop centrifuge with 96-Well Plate Adapters (optional)	MLS (major laboratory supplier)
Harris Manual Punch, 1.2 mm	MLS
BSD600-Duet Semi-Automated Dried Sample Punch Instrument with a 1.2 mm punch head	Contact your local Life Technologies support representative for information.
BSD1000-GenePunch Automated Dried Sample Punch Instrument with a 1.2 mm punch head	
Bode Buccal DNA Collector™	4467893 This part number is not available for sale in the US.
Copan NUCLEIC-CARD™	Contact your local Life Technologies support representative for information. This product is not available for sale in the US.
96 well, deep well plate	4392904

Table 5 Software

Software	Source
3500/3500xL Data Collection Software v2 (RUO)	4475183
HID Updater 3500 Data Collection Software v2	4480670

Software	Source
3130 Data Collection Software v4	4475105
3130*xl* Data Collection Software v4	4475126
3730/3730*xl* Data Collection Software v4	4475154
3130/3730 Data Collection Software v4 6-Dye Module v1	Contact your Life Technologies HID representative
GeneMapper® *ID-X* Software v1.4 Full Installation	4479707
GeneMapper® *ID-X* Software v1.4 Client Installation	4479711

Table 6 User-supplied materials

Item†	Source
GlobalFiler™ Express PCR Amplification Kit, 200 reaction	4476609
GlobalFiler™ Express PCR Amplification Kit, 1000 reaction	4474665
Prep-n-Go™ Buffer (untreated paper)	4467079
Prep-n-Go™ Buffer (buccal swab)	4471406
3130 Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130*xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4® Polymer for 3100/3100-*Avant* Genetic Analyzers	4316355
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10×	402824
Hi-Di™ Formamide	4311320
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
MicroAmp® Optical 96-Well Reaction Plate	N8010560
250-µL Glass Syringe (array-fill syringe)	4304470
5.0-mL Glass Syringe (polymer-reserve syringe)	628-3731
For a complete list of parts and accessories for the 3100 instrument, refer to Appendix B of the *3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (Pub. no. 4335393).	
3130*xl* Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130*xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4® Polymer for 3130/3130*xl* Genetic Analyzers	4352755
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10×	402824

Item†	Source
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
MicroAmp® Optical 96-Well Reaction Plate	N8010560
Hi-Di™ Formamide	4311320
For a complete list of parts and accessories for the 3130*xl* instrument, refer to Appendix A of the *3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (Pub. no. 4352716).	
3500/3500xL Analyzer materials	
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
POP-4® polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4® polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715
For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the *3500/3500xL Genetic Analyzer User Guide* (PN 4401661).	
PCR Amplification	
MicroAmp® 96-Well Tray	N8010541
MicroAmp® Reaction Tube with Cap, 0.2-mL	N8010540
MicroAmp® 8-Tube Strip, 0.2-mL	N8010580
MicroAmp® 8-Cap Strip	N8010535
MicroAmp® 96-Well Tray/Retainer Set	403081
MicroAmp® 96-Well Base	N8010531
MicroAmp® Clear Adhesive Film	4306311
MicroAmp® Optical Adhesive Film	4311971
MicroAmp® Optical 96-Well Reaction Plate	N8010560
Other user-supplied materials	
Hi-Di™ Formamide, 25-mL	4311320
Aerosol resistant pipette tips	MLS
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS

Item†	Source
Tube, 50-mL Falcon	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Vortex	MLS

† For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

C Plate Layouts

Example PCR plate layout

The following layout is recommended for use with the sensitivity experiment on page 19. Create 3 identical plates for amplification at 3 different cycle numbers.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Samp 1	Samp 8	Samp 15	Samp 22								
B	Samp 2	Samp 9	Samp 16	Samp 23								
C	Samp 3	Samp 10	Samp 17	Samp 24								
D	Samp 4	Samp 11	Samp 18	Samp 25								
E	Samp 5	Samp 12	Samp 19	Samp 26								
F	Samp 6	Samp 13	Samp 20	Neg ctrl								
G	Samp 7	Samp 14	Samp 21	007								
H												

Example electrophoresis plate layout

The following layout is recommended for use with the sensitivity experiment on page 19.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Samp 1	Samp 8	Samp 15	Samp 22	Samp 1	Samp 8	Samp 15	Samp 22	Samp 1	Samp 8	Samp 15	Samp 22
B	Samp 2	Samp 9	Samp 16	Samp 23	Samp 2	Samp 9	Samp 16	Samp 23	Samp 2	Samp 9	Samp 16	Samp 23
C	Samp 3	Samp 10	Samp 17	Samp 24	Samp 3	Samp 10	Samp 17	Samp 24	Samp 3	Samp 10	Samp 17	Samp 24
D	Samp 4	Samp 11	Samp 18	Samp 25	Samp 4	Samp 11	Samp 18	Samp 25	Samp 4	Samp 11	Samp 18	Samp 25
E	Samp 5	Samp 12	Samp 19	Samp 26	Samp 5	Samp 12	Samp 19	Samp 26	Samp 5	Samp 12	Samp 19	Samp 26
F	Samp 6	Samp 13	Samp 20	Neg ctrl	Samp 6	Samp 13	Samp 20	Neg ctrl	Samp 6	Samp 13	Samp 20	Neg ctrl
G	Samp 7	Samp 14	Samp 21	007	Samp 7	Samp 14	Samp 21	007	Samp 7	Samp 14	Samp 21	007
H	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank

Cycle 1 Cycle 2 Cycle 3

D

PCR Work Areas

Work area setup and lab design

Many resources are available for the appropriate design of a PCR laboratory. If you are using a GlobalFiler™ Express Kit for:

- Forensic DNA testing, refer to "Forensic Laboratories: Handbook for Facility Planning, Design, Construction and Moving," National Institute of Justice, 1998
- Parentage DNA testing, refer to the "Guidance for Standards for Parentage Relationship Testing Laboratories," American Association of Blood Banks, 7th edition, 2004

The sensitivity of GlobalFiler™ Express Kit (and other PCR-based tests) enables amplification of minute quantities of DNA, necessitating precautions to avoid contamination of samples yet to be amplified (Kwok and Higuchi, 1989).

Also take care while handling and processing samples to prevent contamination by human DNA. Wear gloves at all times and change them frequently. Close sample tubes when not in use. Limit aerosol dispersal by handling sample tubes and reagents carefully.

Note: We do not intend these references for laboratory design to constitute all precautions and care necessary for using PCR technology.

PCR setup work area

IMPORTANT! These items should never leave the PCR Setup Work Area.

- Calculator
- Gloves, disposable
- Marker pen, permanent
- Microcentrifuge
- Microcentrifuge tubes, 1.5-mL, or 2.0-mL, or other appropriate clean tube (for Master Mix preparation)
- Microcentrifuge tube rack
- Pipette tips, sterile, disposable hydrophobic filter-plugged
- Pipettors

- Tube decapper, autoclavable
- Vortex

Amplified DNA work area

IMPORTANT! Place the thermal cyclers in the Amplified DNA Work Area.

You can use the following systems:

- Veriti® 96-Well Thermal Cycler (Part no. 4375786)
- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block

IMPORTANT! The GlobalFiler™ Express Kit is not validated for use with the GeneAmp® PCR System 9700 with the Aluminium 96-Well Block. Use of this thermal cycling platform may adversely affect performance of the GlobalFiler™ Express Kit.

IMPORTANT! The GlobalFiler™ Express Kit is validated for use with the standard Veriti® 96-well Thermal Cycler (Part no. 4375786) NOT the Veriti® 96-Well Fast Thermal Cycler (Part no. 4375305). Please ensure you are using the correct Veriti® Thermal Cycler model.

E

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Specific chemical handling

CAS	Chemical	Phrase
26628-22-8	Sodium Azide	Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

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Documentation and Support

Related documentation

Document title	Part number
GlobalFiler™ Express PCR Amplification Kit Quick Reference – PCR Amplification and CE	4480794
GlobalFiler™ Express PCR Amplification Kit Quick Reference – PCR Setup – Treated Paper Substrate	4480904
GlobalFiler™ Express PCR Amplification Kit Quick Reference – PCR Setup – Untreated Paper Substrate	4480795
GlobalFiler™ Express PCR Amplification Kit Quick Reference – PCR Setup – Swab Substrate	4477601
3100/3100-Avant Data Collection v2.0 User Guide	4347102
3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin	4350218
3100 Genetic Analyzer User Manual (Data Collection v1.1)	4315834
3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® *PCR Amplification Kit PCR Products User Bulletin*	4332345
Applied Biosystems® *3130/3100xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin*	4363787
Applied Biosystems® *3130/3130xl Genetic Analyzers Getting Started Guide*	4352715
Applied Biosystems® *3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide*	4352716
Applied Biosystems® *3130/3130xl Genetic Analyzers Quick Reference Card*	4362825
Applied Biosystems® *3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide*	4359472
Applied Biosystems® *3130/3100xl DNA Analyzers User Guide*	4331468
Applied Biosystems® *3500/3500xL Genetic Analyzer Quick Reference Card*	4401662
Applied Biosystems® *3500/3500xL Genetic Analyzer User Guide, Data Collection v1.0*	4401661
Applied Biosystems® *3500/3500xL Genetic Analyzer User Bulletin: Solutions to issues related to software, data, hardware, and consumables* **Note:** Additional user bulletins may be available at **www.lifetechnologies.com**	4445098
Applied Biosystems® *3730/3730xl Genetic Analyzer Getting Started Guide*	4359476
GeneAmp® PCR System 9700 Base Module User's Manual	N805-0200
Quantifiler® Kits: Quantifiler® *Human DNA Quantification Kit and* Quantifiler® *Y Human Male DNA Quantification Kit User's Manual*	4344790
GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide	4338775
GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial	4335523
Installation Procedures and New Features for GeneMapper® ID Software v3.2 User Bulletin	4352543
GeneMapper® ID-X Software v1.4 New Features and Installation Procedures User Bulletin	4477684
GeneMapper® ID-X Software Version 1.0 Getting Started Guide	4375574
GeneMapper® ID-X Software Version 1.0 Quick Reference Guide	4375670

Document title	Part number
GeneMapper® ID-X Software Version 1.0 Reference Guide	4375671
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Getting Started Guide	4396773
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Quick Reference Guide	4402094
GeneMapper® ID-X Software Version 1.2 Reference Guide	4426481
GeneMapper® ID-X Software Version 1.2 Quick Reference Guide	4426482

Portable document format (PDF) versions of this guide and the documents listed above are available at **www.lifetechnologies.com**.

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Headquarters
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